VOLUME I JUNE, 1908 No. 2

NOTE ON A LIVER ABSCESS OF AMOEBIC ORIGIN IN A MONKEY.

Plate VIII.

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SINCE November 1906 I have had in the animal house attached to the Bacteriological Laboratory, a rather large female *Macacus pileatus* (No. 47). This monkey, which had been bought from a villager, had never been used for any experiment: it was kept in the same cage with a monkey inoculated with yaws. In October 1907 the monkey, which so far had been in good health, began to lose appetite, and looked somewhat ill; nevertheless the animal continued to jump about in the cage and play with its companion till a short time before death, on 5. xi. 07. The monkey never had any diarrhoea, and I thought it might have died from a peculiar form of malaria, extremely common in Ceylon monkeys.

Autopsy: made immediately after death: lungs and heart normal; heart blood and venous blood did not show the presence of Plasmodia or any other blood parasites; spleen not enlarged, contained no pigment and no haematozoa; intestine to all appearances normal; the mucosa did not show any ulceration nor scars due to earlier ulcerations; contents of colon and rectum semisolid, no blood or mucus; liver somewhat enlarged, a yellowish tumour on the upper surface—which I at first took for a cyst. On cutting, it became clear that it was an abscess the size of a very small nut; the pus was yellowish with a little blood and resembled the pus found in human liver abscesses.

On microscopical examination the pus was found to contain leucocytes, a few red corpuscles, and much detritus. What struck me was the presence of a few slowly moving amoebae. The amoebae were more numerous in scrapings from the walls of the abscess. The amoebae were not present in the intestinal contents which appeared to

be quite normal. I may say at once that (microscopically and culturally) the pus of the abscess, the spleen juice, and the blood from the heart, did not show the presence of any bacteria.

Description of the Amoeba. In fresh preparations from the pus of the abscess, the organisms, which were all of large dimensions, appeared moving slowly with the well-known amoeboid movements. The pseudopodia were short, blunt, rather slowly emitted and retracted; the distinction between endosarc and ectosarc was apparently not very marked. The whole body of the parasites appeared vacuolated; but no pulsating vacuoles were present. No nucleus could be distinguished. Some of the amoebae contained red blood corpuscles.

Stained preparations. I stained several films from the pus of the abscess by means of the Romanovsky-Leishman method. The amoebae stain bluish, are oval or rounded, and measure 40 to 70 μ. They occasionally contain red blood corpuscles which appear pinkish or yellowish when stained. In some of the amoebae the nucleus cannot be made out; in others it is small (3 to 6 μ), contains some chromatin, is rounded, and occupies an eccentric position. I have not come across encysted or developmental forms.

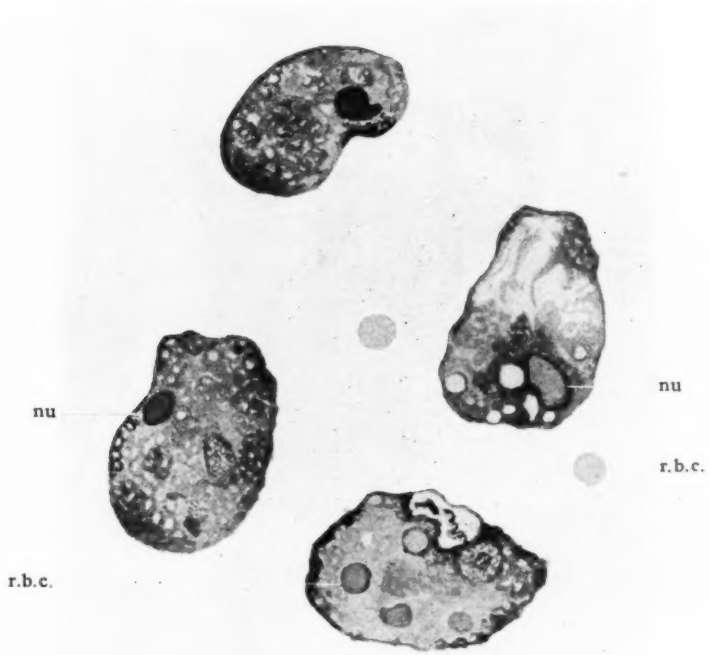
It is probable, from what has been said regarding the appearances presented by the intestines at autopsy, that this monkey had never had dysentery.

I have seen several cases of spontaneous dysentery among monkeys where the intestinal contents contained amoebae which I believe to be identical with the species found in this liver abscess.

This is the first time, however, that I have come across a liver abscess in a monkey. It is to be noted that amoebae may be frequently found in the faeces of apparently quite healthy monkeys, a fact already noted by several observers; these amoebae, however, are morphologically different as they generally present a rather large, distinct nucleus, and are apparently non-pathogenic.

In conclusion, it seems to me that the abscess of the liver found in the monkey was due to the amoebae I have briefly described, as neither the pus of the abscess, nor the spleen juice, nor the blood of the heart, was found to contain any bacteria; either microscopically or culturally.

For this amoeba I propose the name *Entamoeba nuttalli*, after Professor George H. F. Nuttall.



ENTAMOEBA NUTTALLI.

Preparation stained by Leishman's method: nu = nucleus, r.b.c. = red blood corpuscles (several of the latter are contained in the parasite at the bottom of the figure).

ON THE LARVAL AND PUPAL STAGES OF *ANOPHELES MACULIPENNIS*, MEIGEN.

Plates IX and X.

(Continued from *Journ. of Hyg.* Vol. VII. p. 318.)

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(*From the Morphological Laboratory of the University of Cambridge.*)

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PART II. THE LARVA (*continued*).

The Muscular System.

The muscles of the head. In the preceding part of this paper (*Journ. of Hyg.*, 1907, p. 297) some account was given of the muscles of the pharynx and there remain to be considered the rest of the muscles of the head.

These are concerned with the movements of the brushes, the antennae, the mandibles, the maxillae, and the labium.

The muscles of the brushes consist of two pairs, an external and an internal (Figs. 1 and 2). Both pairs arise from the dorsal wall of the head-capsule, and their points of origin coincide with certain symmetrical patches of pigment situated on the surface of the head. Each pair passes forwards to be inserted into the base of the brush of its side. The external muscles (*b'*. *m'*.) take their origin between and in front of the eyes, and are separated from one another by an interval equal to about half the diameter of the head at its widest part. They converge somewhat as they pass forwards, and each muscle is attached to a chitinous apodeme of the epipharynx. The internal muscles (*b.m.*) arise almost in contact with one another in the middle line of the head just behind the eyes. For the greater part of their course they run almost parallel with one another, and are likewise inserted into a pair of apodemes at the bases of the brushes, slightly above and internal to those of the external muscles.

With regard to the working of these muscles, so far as could be ascertained, they appeared to contract simultaneously with the result that the brushes are depressed so that they become directed backwards and inwards and lie within the space enclosed by the mouth-parts, and their setae come in contact with the surface of the epipharynx. When the muscles are relaxed the brushes regain their normal attitude in virtue of the elasticity of the chitinous framework at their bases upon which the strain is exerted.

The rapid backward and forward motion of the brushes can be readily observed, and though as a rule they work simultaneously, at times one brush may work independently of its fellow.

A third pair of muscles, the *epipharyngeal muscles*[1] (*ep.m.* in Figs. 1 and 2), arise from the middle line on the roof of the head-capsule and just behind the points of origin of the external muscles of the brushes. They pass forwards very nearly parallel to each other and are inserted close together into the membrane of the epipharynx behind the points of insertion of the muscles of the brushes. They appear to function principally as elevator muscles to the roof of the mouth.

At the base of each antenna, along its inner aspect, an *antennal muscle* (*at.m.* in Fig. 1) is inserted. It takes its origin from a strongly chitinised apodeme (the tentorium) arising from the floor of the head. The muscle passes obliquely upwards and outwards in its course to the antenna.

[1] These muscles have already been referred to in the account of the pharynx (*Journ. of Hyg.* 1907, p. 299).

The remaining muscles of the head which have yet to be dealt with are those belonging to the mouth-parts (Fig. 2), and the most prominent of them are the muscles of the mandibles.

Each mandible is moved by a pair of stout muscles, an adductor, which is attached to its inner angle, and an abductor attached to its outer angle. The adductor muscle is the stouter of the two and consists of four separate bands of fibres (*a.m.*). Three of these arise from the wall of the head-capsule a short distance behind and below the eyes, but the fourth band arises separately at a point somewhat in front of the places of origin of the former, and, moreover, it is ventro-lateral in position. The four bundles pass upwards and inwards, converging as they do so, and are inserted into a stout chitinous process from the mandible. The abductor muscle similarly consists of four distinct bands or bundles of fibres (*a'.m'.*), two of these arise close to the three dorsal bands of the adductor while the other two take their origin near to the ventral bundle of the latter.

Attached to about the middle of the base of each maxilla are a pair of muscle bands (*mx.*). If these be traced backwards they are seen to run in close proximity to each other, and their points of origin are situated ventro-laterally on the cranial wall, near to those of the ventral bands of the abductor muscle of the mandible.

The labial plate is provided with a pair of depressor muscles; they are attached on either side near to the base of that organ where it becomes confluent with the ventral surface of the cranium, and converge as they pass forwards to their points of insertion.

Owing to the imperfect transparency of the head-capsule it has not been possible to fully study the movements of the various muscles in the living larva. Their functions, as implied by the names here given, have been largely inferred from a study of their relations to the different parts principally by means of serial sections.

The muscles of the thorax. In the thorax there is a well developed system of longitudinal muscles arranged in a dorsal and a ventral series. The dorsal series, or longitudinal tergal muscles, lie a short distance beneath the integument and occupy much of the mid-dorsal area above the digestive canal. The ventral series, or longitudinal sternal muscles, form two groups, one on either side of the nervous system. Immediately in front of the prothoracic ganglia, a transverse muscle band crosses over the nerve cord and unites together certain of the longitudinal sternal muscles. Tergo-sternal muscles are also present, and the most anterior of them is a stout muscle which takes origin from

the dorsal integument in the prothoracic region on either side. This muscle passes downwards and outwards, and becomes attached to the ventral body wall, just external to the imaginal prothoracic leg-bud of its side. About the middle of the thorax a second series of such muscles are present; they consist of several bundles, widely separated from one another, which pass downwards and inwards to the sternal region. In the hinder part of the thorax a third series of tergo-sternal muscles are present; they pass almost vertically downwards on either side between the salivary glands and the cardiac coeca.

The musculature of the thorax exhibits clear indications of an arrangement into three segments, and it is especially well seen in connection with the longitudinal sternal muscles.

In the fully grown larva the developing imaginal thoracic muscles form very prominent objects; they are disposed in a longitudinal and a vertical series on either side of the body. They are very deeply staining and are in the form of short bundles in which are distributed great numbers of minute nuclei, but they do not exhibit any cross striation. The longitudinal series lie above the larval longitudinal tergal muscles and take the form of parallel cords situated on either side of the middle line. The vertical series are situated immediately external to the salivary glands, and are arranged in an anterior and a posterior group on each side.

A number of muscles are also found in the neck, and their action brings about the remarkable rotatory movements of the head. The smaller of these muscles lie for the most part in the neck itself and pass in an oblique manner from one side to the other. There are, in addition, some much stouter muscles, which arise from the prothoracic region and pass up through the neck to be inserted into the base of the head.

The musculature of the abdomen. In the abdomen there is no segmentally repeated system of tergo-sternal muscles, such as is present in many insects. The longitudinal muscles[1], however, are very well developed and are much stouter than those of the thorax, and it is due to the action of these muscles that the larva performs its vigorous eel-like movements as it swims tail foremost through the water. The longitudinal tergal muscles are grouped together on either side of the dorsal vessel, and the sternal series have similar relations with the

[1] Grandpré and Charmoy remark (p. 33), "Les principaux muscles sont les muscles longitudinaux et circulaires de l'abdomen"; this statement, however, is incorrect as there are no circular muscles present.

nervous system (Fig. 3). In the eighth and ninth segments, the disposition of the longitudinal muscles is much modified, and many of the muscles come to lie nearer the digestive canal.

Just in front of each nerve ganglion, a transverse band crosses the nerve cord, and passes along the line of junction of two adjacent segments (Fig. 22).

In the eighth abdominal segment there is a special system of muscles in connection with the supporting skeleton of the spiracles. In order to attempt an explanation of these muscles it is necessary to briefly consider first the structure of this skeleton. From the posterior region of this segment of the body a median spiracular lobe, as it may be termed, projects backwards and very slightly upwards (Fig. 4). It stands out free from the body, and is supported basally by a special chitinised sclerite (*sc.*), which is prolonged on either side into a prominent plate provided with large backwardly directed teeth (*pl.*). The plates of either side are connected together by means of a transverse chitinous band (*t.b.*) which passes beneath the median spiracular lobe, and serves also to maintain the latter in a position so as to project out freely from the general surface of the body. The lobe itself consists of two thin lateral plates (*c.pl.*) which have their free margins curved upwards and inwards, and a central, oblong plate of dark chitin with a curiously sculptured surface (*m.pl.*). At the apex of the lobe the two lateral plates are joined together by means of a median transverse plate (*t.pl.*). The two spiracles (*sp.*) are situated at the anterior end of the median lobe near to the base of the latter and where it becomes joined to the segment. In front of and between the spiracles is situated a moveable transverse plate standing more or less at right angles with the general surface of the body (*f.pl.*). This plate is attached to a stout hollow peg of very dark chitin (*p.*) which projects beneath the integument slightly into the cavity of the animal. To this same peg is also attached the median plate (*m.pl.*) of the spiracular lobe.

There are three paired sets of muscles in connection with the respiratory apparatus as follows: firstly, a pair of longitudinal muscles (*l.musc.*), which take their origin from the median peg (*p.*), and pass directly backwards to be inserted into the spiracular lobe, in the mid-ventral line near to the apex of that organ. Secondly, a series of muscles (*i.mus.*) arise from the ventro-lateral margin of the eighth abdominal segment and, pursuing a backward and inward course, they converge and pass between the two longitudinal muscles just referred

to. The two series cross one another (Fig. 5) and are inserted into the median plate (*m.pl.*) of the spiracular lobe along its middle line. Thirdly, a pair of stout muscles arise one on either side close to the imaginal bud of the gonapophysis of its side and slightly dorsal to it (*o.mus.*). These muscles pass backwards and upwards in an oblique direction, and just external to the longitudinal muscles, and are inserted on either side of the median area (*m.pl.*).

When the animal dives suddenly down into the water, the upper surface of the spiracular lobe is converted into a kind of cup which retains, as a rule, a bubble of air. The contractions of the two sets of muscles (*i.mus.* and *o.mus.*) depress the floor of the lobe, with the result that the sides of the organ are curved inwards, and brought much closer together, and, furthermore, by the action of the longitudinal muscles (*l.musc.*), the anterior and posterior portions of the organ similarly become drawn closer together, and in this way a temporary cup is formed. When the muscles are relaxed, the organ spreads itself out flat, and forms a plate which helps to support the larva at the surface of the water.

In the ninth abdominal segment, a pair of stout muscles take their origin one on either side of middle line of the tergum, and pass obliquely downwards and backwards to become attached, one on either side, to the base of the skeleton which supports the ventral fan of hairs (Fig. 6). By means of the contractions of this pair of muscles the ventral fan, or "tail-fin," can be moved to one side or the other as occasion may demand and, in this way, it appears that the fan of hairs functions as a kind of rudder which steers the animal as it is swimming. If this analogy be a correct one, the two muscles may be regarded as being comparable with the tiller cords attached to the rudder of a boat.

The Fat-body.

The fat-body is almost entirely confined to the thorax and the first seven abdominal segments. It consists of a parietal layer situated just beneath the integument, a visceral layer lining the body-cavity, and lying between the longitudinal muscles and the gut-wall, and a peritracheal layer which invests some of the principal tracheal trunks. The limits of distribution of each of these divisions vary according to the age of the larvae, and a certain amount of individual variation is also noticeable among specimens of as near as possible the same age.

The visceral layer of the fat-body (Figs. 3 and 9, *f.b.*), although but a thin sheet of tissue, is very conspicuous, as its cells are loaded with a

mass of minute dark olive coloured granules which are very refractive. It is in this layer that metabolism appears to be most active, for the granules are much less abundant in other parts of the animal. A good deal of the greenish colour of the larva is due to the fat-body shining through the transparent integument. The fat-body cells are much vacuolated, and the cell boundaries are only to be distinguished with considerable difficulty. Their nuclei are for the most part small and inconspicuous, and are irregularly stellate in form. The protoplasm of the cells contains, in addition to the granules just mentioned, a number of clear spherical globules which stain very readily with Orange G. The structure of the fat-body agrees in all respects with Berlese's description of the same tissue in the larva of *Culex spathaepalpis* Rond, and he states that the globules are of an albuminoid nature (1901, p. 98, and Tav. V, Fig. 60).

The *sub-hypodermal cells* have the general characters of fat-body cells and, moreover, their protoplasm contains similar granules. As already stated in the previous part of this paper (*Journ. of Hyg.*, 1907, p. 296) Viallanes concludes that they come under the category of this tissue.

The various areas of the fat-body are not to be regarded as being quite separate and distinct from one another. The divisions here adopted have been used because they appear to be convenient for descriptive purposes, and in certain parts of the body one layer merges into the other. The parietal layer is interruped at each segment, and in this respect shows a metameric arrangement. The visceral layer, however, is in the form of a continuous sheet passing directly from one segment to another.

The Reproductive Organs.

The rudiments of the gonads differ in form in the two sexes, the male rudiment being short and somewhat globular, while that of the female is relatively longer and fusiform.

The male rudiment in a larva measuring 2·5 mm.[1] in length (Fig. 14) consists of a germinal region, a long anteriorly directed terminal filament or, so-called Müller's thread, and a slight rudiment of the future generative duct. The organ is situated in the sixth abdominal segment. In larvae measuring 5·5—7·5 mm. long, the proportions of these three regions have undergone considerable altera-

[1] The measurements are taken from the tips of the brushes to the extremity of the abdomen, and do not include the dorsal tuft of setae on the ninth segment.

tion, and a long rudiment of the future reproductive duct has been developed. It extends backwards nearly to the posterior border of the seventh abdominal segment (Fig. 11). The duct, at first, takes the form of a solid cellular strand, but becomes hollowed out at a later period. The terminal filament is embedded in the visceral layer of the fat-body (Fig. 13 *a*.).

In a larva measuring 6·75 mm. long, the female rudiment occupies both the fifth and sixth abdominal segments (Fig. 12), it is more elongated than that of the male of a corresponding age, and shows marked differences in its histological characters. In both sexes the genital rudiment is invested exteriorly by an apparently structureless tunic, and in the later larval periods the central mass of the organ is seen to consist of a number of small cell-clusters, separated from one another by a small amount of interstitial tissue. It is from these cell-clusters that the spermospores in the case of the male and the ovarian follicles in the female become developed (Figs. 12 and 13 *b*.).

But little is known concerning the development of the reproductive organs of Diptera, or other Insecta, during the larval period. Among the Brachycera, some observations are given by Weismann (1864, p. 205, Taf. XIV, Figs. 67—72) in the case of *Musca vomitoria* and *Sarcophaga carnaria*. In the Nemocera, the larval gonads are best known for *Chironomus* (Balbiani, 1885, p. 527, and Miall and Hammond, 1900, p. 135), and *Corethra* (Weismann, 1866, p. 99, Taf. VI). Miall and Shelford also give some brief notes on those of *Phalacrocera* (1897, p. 35), and Lécaillon for the female rudiments in *Culex* (1900, p. 96).

In *Chironomus* and *Cecidomyia* it is known that the sexual germs develop at an extremely early period in the embryonic life, being formed as polar cells at the surface of the egg before the blastoderm has been developed. How far this precocious development of the sexual germs is at all general among Insects is at present unknown.

The Nervous System.

The nervous system consists of the brain or cerebral ganglia, and a ventral nerve chain of twelve ganglia (Fig. 23).

The brain alters considerably in shape and in the relations of the parts, during the growth of the larva, especially with regard to the optic and antennary lobes. In the very young larva, the optic lobe is separated from the antennal lobe by a wider area than is the case in

the late larva and, as development progresses, they become as it were drawn closer together.

In its general structure, the brain consists of an outer sheath of ganglion cells, which are thickly massed together in the optic and antennal lobes, and an inner central area consisting for the most part of nerve fibres. Paired nerves are given off supplying the antennae and larval eyes, together with a frontal nerve, which expands into a frontal ganglion lying anterior to the brain and just above the dorsal pharyngeal muscles. The antennal nerve passes obliquely outwards, and enters the imaginal antennal bud where it terminates (Fig. 20). According to Miall and Hammond a similar condition is found in the *Chironomus* larva and in their book on that Dipteron (1900, p. 130) they remark that the imaginal antenna encloses the larval antennary nerve.

The ventral nerve chain comprises the sub-oesophageal ganglion, three thoracic ganglia, and a series of eight ganglia in the abdomen. The sub-oesophageal ganglion innervates the mandibles, the maxillae, and the labium, and is to be regarded as a complex ganglionic mass exhibiting no traces of the primitive ganglia of which it is composed, but its paired structure is noticeable in sections (Fig. 32). Histologically, it consists of an external investment of ganglion cells enclosing a central medullary mass of nerve fibres, which is directly prolonged outwards into the lateral nerves and connectives. The ganglion cells are principally congregated along its anterior, ventral, and lateral borders and are much less numerous on its dorsal aspect. A structureless coat of connective tissues invests the exterior of the ganglion. Posteriorly, a pair of stout nerve cords are given off which pass through the occipital foramen and unite with the first thoracic ganglion. The remaining ventral ganglia have a very similar histological structure, and consist of an outer layer of ganglion cells which enclose an inner core of nerve fibres, and the whole is invested by a sheath or epineurium. The connectives, though they are in reality double, appear to consist of a single cord. The thoracic ganglia are situated close together, the intervening connectives being very short, and the abdominal ganglia are placed anteriorly in their respective segments near to the line of junction with the segment in front (Fig. 22). In the eighth segment the ganglion is larger than those of the preceding segments, it is somewhat triangular in form, and it gives off pairs of nerves which supply both the eighth and last segments. It is therefore to be regarded as a fusion of the two primitive ganglia of

those segments. Each ganglion of the ventral nerve cord gives off a pair of principal nerves to its segment, and they pass outwards between the longitudinal sternal muscles and the integument (Figs. 3 and 22).

A system of transverse nerves is also present; a fine nerve runs along the junction between each pair of segments above the abdominal nerve cord, and each is connected with the ventral ganglia by means of median nerves. Owing to lack of opportunity at present descriptions of this and several other important features in the *Anopheles* larva are postponed for a future paper.

Circulatory System.

The heart is a muscular tube which extends from the posterior margin of the eighth abdominal segment, and passes forwards through the thorax into the head, where it terminates in the neighbourhood of the brain. In the region of the abdomen it consists of a series of consecutive chambers, each being provided with a pair of lateral ostia or inlets and, associated with them, are the alary muscles. In the thorax the heart is much reduced in diameter, there are no alary muscles, and it forms a narrow well-defined tube which is usually termed in Insects the aorta. The latter extends forwards through the occipital foramen and becomes enclosed just behind the brain in an irregular mass of tissue which forms a kind of supporting collar or *anneau de soutien.*

The abdominal portion of the heart consists of a series of eight successive chambers and it expands somewhat at its posterior extremity, the last chamber forming a slight bulbous enlargement. The intimate connection that exists between the tracheal system and the terminal region of the heart has already been referred to (*Journ. of Hygiene,* 1907, p. 311, Pl. V).

The structure of the wall of the heart is entirely muscular, and consists of flattened cells whose protoplasm mainly takes the form of striated fibrillae, which are disposed more or less parallel to one another (Fig. 30). Both externally and internally, the heart is invested by a delicate transparent membrane which is to be regarded as the sarcolemma of the muscle cells (Fig. 29). The striated fibres encircle the heart posteriorly in a slightly oblique manner, but further forwards they become arranged in a longitudinal direction so that their cut ends are only visible in transverse section (Fig. 15). The nuclei of the cells are situated laterally and form noticeable bulgings in the heart wall

(Figs. 9 and 15)[1]. In a transverse section only two of the cells are visible at any given place: they are crescentic in form and are united with each other along the dorsal and ventral lines.

The lateral inlets or ostia are situated in pairs opposite one another on either side of the heart (Fig. 10). Each ostium is formed by a pair of enlarged cells which surround the aperture and are noticeable in stained preparations by their nuclei.

The alary muscles (*al.m.* in Figs. 9 and 10) are markedly cross-striated: each muscle takes its origin from the integument on either side and, as it approaches the heart, it spreads out fanwise and divides and sub-divides so as to form a network of fine fibrillae. These fibrillae are arranged in the form of a dorsal and ventral sheet (Fig. 9), and in the space or interval left between them are situated the pericardial cells. In addition to the alary muscles, the heart is maintained in position by means of delicate strands of connective tissue placed at different points.

The pericardial cells (Figs. 9 and 10) are placed laterally in relation to the heart, and form a chain of cells arranged in a linear series on either side. The cells are disposed with their long axes parallel with the length of the body, but the chains are not continuous throughout their length, numerous gaps or breaks in the series being present. They extend right through the abdomen to about the middle of the eighth segment. The cells contain several nuclei and their protoplasm is much vacuolated. In *Sayomyia* the pericardial cells were described by Wagner (1835) as "piriform bodies" and later by Dogiel as "apolar nerve cells" (1877, p. 9). In the anterior part of the thorax, and situated close to the heart on either side, is a longitudinal cord of multinucleated cells (Fig. 15). These two cords occupy very much the same position as the pericardial cells in the region of the abdomen and very possibly they are structures of a similar nature.

The aorta is a forward prolongation of the heart through the thorax into the head. It has stout walls, and the nuclei of the cells are disposed with long axes parallel with the length of the tube. No alary muscles are present. The aorta passes forwards between the two dorsally placed cardiac coeca, and lies just over the oesophagus but, as it approaches the occipital foramen, it comes to lie nearer the dorsal integument. Just behind the brain, it becomes supported by an irregular mass of cells which envelops it both ventrally and laterally.

[1] In the larva of *Sayomyia* the nuclei project a long way inwards and were termed the "stalked cells" by Leydig.

This structure is a kind of supporting collar (*anneau de soutien*) and is composed of cells which have no definite outlines and are in some cases vacuolated. It is invested by a connective-tissue coat, and is maintained in position by some delicate muscle fibres arising from the head-capsule. Furthermore, it is also attached by means of fine membranous strands both to the connective-tissue coat of the oesophagus and, a little further forwards, to the commissure uniting the two cerebral ganglia (Figs. 17, 18 and 21). Posteriorly, the organ bifurcates into a pair of cellular prolongations (Fig. 16); while anteriorly it extends a short distance forwards between the cerebral ganglia. A similar structure has been described by Weismann (1864, Pl. VIII, Fig. 8) in the larvae of Muscids and also by Lowne (pp. 88 and 91). It is, furthermore, found in the larvae of the Syrphidae (Kunckel de Herculais, 1875, Pl. XIII, Fig. 5), of the Tachinid *Thrixion* (Pantel, 1898, p. 170) and of *Sayomyia* (Dogiel, 1877, Fig. 1). In the latter it is placed behind the brain as in *Anopheles*, but in *Thrixion* it lies for the most part above the cerebral commissure and in a more forward position. Dogiel states that in front of the collar the aorta divides into two lamellae, one of which extends under the brain towards the eye, and the other is more dorsally situated. At the point where the two lamellae arise the aorta opens freely into the haemocoelic space of the head. In *Anopheles* the further course of the aorta was found to be very difficult to follow, but it seems to divide into two lamellae as in *Sayomyia*, one of which is closely applied to the under surface of the cerebral commissure, and the other appears to be in intimate association with the dorsal surface of the anterior prolongation of the *anneau de soutien*.

The dorsal lamella in *Sayomyia* and *Anopheles* appears to be the homologue of the *gouttière susoesophagienne* described by Pantel in the heart of the larva of the Tachinid genus *Thrixion*.

The Oenocytes.

Oenocytes are present in the larva of *Anopheles* and belong to two varieties—the large and the small. The large oenocytes (Fig. 26) are segmentally arranged in clusters, and are present in each of the first seven abdominal segments, but were not observed either in the eighth or ninth segments, and they are likewise wanting in the thorax. In each of the segments where they occur, they consist of two pairs of very large cells on either side, which are situated a short distance beneath the hypodermis and towards the posterior margin of the

segment. Each cluster of these cells is ventro-lateral in position. The cells are rounded or somewhat oval in form with a granular and very deeply staining cytoplasm, and each cell is bounded by a clearly defined membranous investment. Numerous large vacuoles are generally present and when viewed in sections they give the cells the appearance of being penetrated by intra-cellular canals. Each oenocyte has a very prominent nucleus containing chromatin strands situated towards the periphery, and a deeply staining nucleolus is also present.

The small oenocytes are very numerous and, moreover, have no definite arrangement though they are sometimes found in pairs (Figs. 22 and 27). They occur just beneath the hypodermis in the neighbourhood of each group of the larger oenocytes, and are mainly situated anterior to the latter, but they also occur in some numbers along the floor of each segment on either side of the nerve cord. They have a similar marked affinity for staining reagents and frequently each contains a clear vacuole (Fig. 22). A nucleus with a central nucleolus is present, and these cells are easily distinguished from the surrounding fat-body by their definite outlines and their staining properties. They occur in the first seven abdominal segments, a few are also present in the eighth but none were to be discovered in the last segment.

The embryonic development of the oenocytes has been studied by Graber, Heymons, Heider, and Wheeler. They arise by a proliferation of the ectoderm just behind the tracheal invaginations, and similar metameric cell-clusters have also been observed by Tichomiroff in the embryo of the "silkworm."

Wielowiejski (1886) was the first to devote special attention to these cells and the name oenocyte is due to him. He pointed out their resemblance to the blood corpuscles, to the fat-body, to the pericardial cells, and to the light-producing tissue of the phosphorescent organs and classified these different elements together under the category of blood tissue. In his paper he describes the oenocytes in various orders of Insects, but unfortunately his account is not accompanied by any figures. Among the Culicidae, he refers briefly to their occurrence in the larvae of *Sayomyia* (*Corethra*) *plumicornis* and *Culex pipiens*. In the former he mentions that they are of two kinds, the large and small oenocytes, and that they are situated between the cells of the outer layer of the fat-body. They are restricted to the abdominal region and are collected together into segmentally repeated groups (1886, pp. 516—17). In the *Culex* larva he describes their occurrence as follows: "Die kleinen Oenocythen liegen auf der der Leibeshöhle

zugewandten Fläche des äusseren Lappens[1] festgeklebt, oftmals bis 10 und mehr auf einem Querschnitte und zeichnen sich durch grosse Tinctionsfähigkeit vor sonstigen aus. Grosse Oenocythen sind auch in Gruppen zu vier bis fünf segmentweise im Abdomen angeordnet, liegen aber eben so wie die kleinen auf der Innenseite des die Leibeshöhle auskleidenden Fettkörperlappens befestigt."

Oenocytes have been discovered in a number of dipterous larvae, and it is extremely probable that they are of general occurrence throughout the order. In *Chironomus* they have been described by Wielowiejski (p. 515) and Miall and Hammond (1900, pp. 40—42). The large oenocytes are restricted to the abdomen, and consist of a group of five very large cells on either side in the first eight segments. The fifth cell in each group lies in front of the cluster of four cells and, moreover, differs from them in containing two nuclei, viz. a large central one and a small one situated at the periphery. The small oenocytes are very numerous and are found towards the ventral surface of the metathoracic and abdominal segments, just beneath the integument. Oenocytes are also present in the larvae of *Tipula oleracea* (Wielowiejski), *Phalacrocera replicata* (Miall and Shelford, 1897, pp. 352), *Simulium* (Vaney, 1202) and other Nemocera.

Among the Brachycera they appear to have been very little studied. Wielowiejski (p. 520) states that in this group of Diptera they present the characters of the large oenocytes of *Chironomus*, and differ chiefly in being more numerous, and in having a more intimate connection with branches of the tracheae. They have been carefully studied, however, by Pantel (1898, p. 210) in the Tachinid, *Thrixion halidayanum*. In the larva of this fly they are collected into a ventral and a lateral group on each side, in the seven posterior segments of the body. Each group contains as a rule 3–6 cells, which are soldered together into a "chapelet." Pantel puts forward the suggestion that possibly one of the two groups in *Thrixion* is the homologue of the binucleated cell in *Chironomus* and that the latter is in reality the rudiment of a colony of uninucleate cells.

Among the Pupipara, according to Berlese (1901, p. 145) in *Melophagus ovinus*, the oenocytes are disposed in metameric groups in the larva but, at the commencement of nymphosis, they multiply and are found in numbers among the cells of the fat-body, and subsequently disappear at the time when the Malpighian tubes are developing.

[1] i.e. the "Fettkörperlappens."

Oenocytes have been found in almost all orders of Insects, but practically nothing is known regarding their function. Wielowiejski believes that they secrete some unknown substance into the blood, and Anglas (p. 405) is of a similar opinion. On the other hand, Pantel concludes from the fact that they absorb methylene blue very rapidly, like the cells of the Malpighian tubes, that their function is that of excretion. Berlese (in *Melophagus*) concludes that they are urinary or excretory cells from the fact that they become free during nymphosis, at a time when the Malpighian tubes are non-functional, and that they work their way among the cells of the fat-body, &c. in order to remove the products of metabolism from them. After a while the oenocytes disappear, and it is at this period that the Malpighian tubes are fully developed.

Koschevnokov (1900) from his studies on the honey bee also concludes that they are urinary cells.

The Imaginal Buds.

The larva of *Anopheles* forms an excellent subject for the study of the imaginal buds since they occur in this type in a very generalised condition. It is, however, beyond the scope of the present paper to deal with this subject in any detail as it needs a much more prolonged study than I have been able to give to it.

Swammerdam appears to have been the earliest observer to correctly interpret certain of the imaginal buds, and he discovered those of the wings and legs. *Culex* was among the types he studied, and since his time Weismann (1866) has studied the imaginal buds of the larva of *Sayomyia*. A few unpublished observations on those of the former genus were made by Hurst, and are mentioned by Miall and Hammond (1892), and Thompson has described the buds of the mouth-parts in that same genus (1905). I am not aware that there are any further accounts which deal with these structures among larval Culicidae.

I. *The imaginal buds of the head.* The largest and most prominent of the head buds are those belonging to the antennae. The *antennal buds* (Figs. 2 and 20) are placed at the bases of the larval antennae, but are not in any way enclosed by the latter and are, moreover, rather deeply situated within the head. In an *Anopheles* larva about three-fifths grown each imaginal antennal bud is a well developed structure and, owing to the growth of the organ within a limited space, the basal joints are somewhat folded and telescoped into one another. As the appendage enlarges, its outer sheath or peripodial membrane

(*pp.m.*) becomes a good deal stretched and is reduced to a thin layer of tissue. At this stage the first three basal joints are already developed but the remainder of the organ is still unsegmented. In a fully grown larva the second joint is greatly enlarged, especially in the male, and its cellular wall is very much thickened so as to almost obliterate the cavity of the joint. This condition is a stage in the development of the prominent antennal sense organ known as Johnston's organ. Although the antennal buds are situated the deepest below the hypodermis of all the imaginal buds, they retain their connection with the surface of the head by means of a short canal which is the persistent mouth of the early invagination.

The buds of the mouth-parts. The earliest indications of the formation of the future mouth-parts are noticeable as special thickenings of the hypodermis, situated at localised points beneath the cuticle of the larval mouth-organs. Of these buds, the rudiments of the future labium, labrum, and maxillary palpi alone attain the condition of imaginal folds prior to pupation. Those of the mandibles and the maxillae remain in the condition of simple, hypodermal thickenings, but are easily recognisable on account of their great affinity for staining reagents. The invaginations of the first mentioned series of buds are superficial in position, and they maintain their communication with the surface by means of wide mouths or openings. The bud of the labrum is unpaired and situated in the middle line. It commences as a hypodermal thickening on the roof of the head and gradually elongates and comes to lie some distance backwards. Those of the maxillary palpi are small structures, and are situated at the bases of the same organs in the larva. The labial bud is extremely prominent (Fig. 31) and takes the form of a paired structure projecting from the bottom of a wide pocket. It is the earliest to appear of the imaginal buds that go to form the mouth-parts. Its first indication in the young larva is in the shape of a thickening of the hypodermis underlying the anterior border of the labial plate. In longitudinal sections through the head of a fully grown larva, the labial buds form a prominent pair of hollow pointed projections which are confluent only at their bases.

II. *The thoracic buds.* In the thoracic region three successive pairs of buds are present on either side belonging to the pro-, meso-, and metathorax respectively. They are disposed in a dorsal and ventral series, the latter eventually giving rise to the three pairs of legs, while of the former series the first pair form the pupal respiratory siphons, the second pair the future wings, and the third pair the halteres. All

the thoracic buds are relatively very large in size, and are superficial in position, lying just beneath the integument. At first they each have the structure of simple buds (Fig. 19) of thickened hypodermis, enclosing a small amount of "mesenchymatous" tissue, but, by the time the larva has become fully grown, they are seen to exhibit a high degree of specialisation. The dorsal prothoracic buds have become the completely formed respiratory trumpets; those of the mesothorax are highly complex organs (Fig. 25), and are much folded and plicated. They already exhibit a wing-like character, the hypodermal cells being greatly drawn out at right angles to the surface of the bud, assuming a pillar-like form, and the upper and lower hypodermal layers meet and fuse with one another. In places they are excavated into hollow tube-like spaces which are forerunners of certain of the future nervures; the dorsal metathoracic buds remain in a more generalised condition, being the smallest and least advanced of the series. The leg-buds are at this period long tube-like organs which are bent upon themselves.

III. *The imaginal buds of the abdomen* comprise a dorsal and ventral pair situated on either side, near the hinder extremity of the body. The dorsal pair of buds form the pupal tail fins; they are placed one on either side of the eighth abdominal segment, and lie within the cavity of the supporting skeleton of the larval spiracles (Figs. 7 and 8). They exhibit very much the same structure as the buds of the wings, the hypodermal cells being enormously drawn out at right angles to the surface of the organ and the protoplasm reduced to narrow strands in which are distributed extremely small nuclei. They are greatly folded, on account of being confined within a limited space, and investing the free surfaces of the folds is a well developed cuticle. They are situated within the lateral plate (*sc.*) of the spiracular skeleton, and project freely backwards reaching to the transverse chitinous band (*t.b.*), and thus lying beneath the spiracular lobe. They appear to differ from the other buds in being directly formed by modification of the hypodermis, without the latter being previously invaginated to form a pocket, and from the bottom of which the buds arise usually as finger-like evaginations. On this account, the buds of the pupal fins are not enclosed within an outer wall or peripodial membrane.

The second pair of buds (Fig. 28) are ventral in their position, and placed close to the points of origin of the outer vertical muscles (*o.mus.* in Fig. 5) of the spiracular apparatus. These buds like the rest lie immediately beneath the hypodermis, and maintain a free communication outwards by means of the mouth of the original

invagination. They are destined to form the gonapophyses or accessory copulatory organs of the imago.

The following table shows the general conditions of the imaginal buds among the Diptera, and the relationships of the Culicidae in this respect to other forms.

Imaginal buds of larval origin.

A. Imaginal buds superficial in position being situated just below the hypodermis; the primitive invaginations remain permanently open.

1. *Anopheles*; *Culex* (Miall and Hammond ex mss. Hurst). Head folds shallow, not extending into larval thorax.

2. *Corethra* (Weismann); *Simulium* (Weismann, Vaney); *Tanypus* (Vaney). Intermediate between 1 and 3.

3. *Chironomus* (Miall and Hammond); *Ceratopogon* (Miall and Hammond); *Stratiomys* (Vaney)?; *Cecidomyia* (Marchal). Head folds extend into larval thorax.

Imaginal buds for the most part of embryonic origin.

B. Thoracic buds superficial in position, cephalic buds as in *C.*

Melophagus (Pratt). Possess an additional pair of ventral cephalic buds not discovered in other Diptera.

C. Imaginal buds deeply situated below hypodermis, only maintaining their connection with the latter by means of a thread-like pedicle. Cephalic buds situated in larval thorax.

Muscids (Weismann); *Anthomyia* (Ganin); *Volucella* (Kunckel d'Herculais); *Eristalis* (Wahl); *Gastrophilus* (Vaney).

The Eyes.

In newly hatched larvae, and in larvae measuring up to 2·75—3·5 mm. in length, a single oval deeply pigmented eye-spot is present on either side of the head. These *larval eyes* ("Nebenaugen") are placed about half way between the neck and the bases of the antennae, and each has its long axis disposed transversely to the length of the head (*l.e.* in Fig. 24 *a* and *b*). In larvae exceeding that length a second type of eye commences to develop, and is first noticeable in the form of a few small rounded pigmented patches situated above, and slightly behind, the larval eyes. These pigmented areas increase in number with the growth of the larva, and the newly added elements can be distinguished

by their nut-brown colour, whereas the already existing ones, owing to their greater amount of pigment, appear dense black (Fig. 24 *a*). These are the rudiments of the future imaginal compound eye ("Hauptauge"). As development proceeds, each of the small pigmented elements increases in size, with the result that it becomes in close contact with its neighbours, and in a larva about 7 mm. long they collectively form a conspicuous black crescentric shaped tract on either side of the head (Fig. 24 *b*). The subsequent extension of the organ is effected by new elements being added around its periphery.

A short optic nerve is present on either side in relation with the larval eye; in larvae about 7 mm. long a nerve from the imaginal ocular rudiment is distinguishable, and the two nerves of a side combine to form the main optic nerve (*b.* and *c.*).

When viewed in sections, the larval eye consists of a central densely pigmented area bordered by elongated cells whose narrower inner ends are directed towards the centre of the organ (*c.*). The eye is densely loaded with pigment of a rust-brown colour which appears black when viewed in thick layers.

The developing imaginal eye consists of a number of deeply pigmented fusiform bodies, placed at right angles with the surface of the head. These elements are the ommatidia of the compound eye, and they each consist, as seen in transverse section, of a central axis or rhabdom surrounded by a circlet of densely pigmented cells (the retinulae). Intervening between the ommatidia and the external cuticle is a layer of but little modified hypodermal cells (*h*). It is destined to form on the outside the corneal facets of the imaginal eye, and on its inside the crystal cells.

The cells of the hypodermis immediately bordering on the imaginal ocular rudiment are markedly columnar in form, and in places it is folded or invaginated into small pit-like areas, which are the very early stages in the development of new ommatidia.

In addition to the Culicidae, certain other families of nemocerous Diptera, having eucephalous larvae, are remarkable in that the imaginal eye develops in close relation with that of the larva and at an early stage in the larval life-history. A short comparative study of the eyes, in both the larvae and pupae of such Diptera, has recently been made by Zavřel (1907).

General Remarks on the Larvae of the Culicidae.

The larvae of the Culicidae may be defined as being aquatic, eucephalous, and with the first three post-cephalic segments fused together to form an evident and greatly enlarged thoracic mass. Although there is but little difficulty in identifying typical Culicid larvae, the greatly enlarged thorax affords the most evident *constant* morphological character, which is common to all the larvae of the family, and by means of which they may be readily separated from those of the allied Nemocerous families Dixidae, Psychodidae, Tipulidae and Chironomidae. It is true that, in the latter family, the first three post-cephalic segments become greatly swollen towards the end of larval life, in consequence of the developing imaginal organs contained within them. In the larval Chironomidae, however, there is no fusion into a compact thorax though the limits between the second and third segments may become partially obliterated. That the thorax of the Culicid larvae is composed of the first three post-cephalic segments is evident from the fact that it contains three of the ventral nerve ganglia, together with three double series of buds of the imaginal thoracic appendages, and moreover its musculature retains to some extent a trisegmental arrangement; externally, however, its composition is not by any means obvious. In *Corethrella brakeleyi* Coq. according to Johannsen (p. 401, pl. 40), the three segments can be perfectly clearly made out, since they have not undergone the same degree of fusion seen among other Culicid larvae. As a rule, the only external indication is to be seen in the principal hairs being arranged in three main transverse series. In all Culicid larvae the abdomen consists of nine segments.

Three principal types of modification are to be seen among Culicid larvae, viz.

(1) The *Anopheles* type where the tracheal system communicates with the exterior by means of a pair of spiracles situated on the dorsal aspect of the eighth abdominal segment, and the respiratory siphon is not developed. Palmate hairs are present on a variable number of the abdominal segments[1], and the comb scales are replaced on either side by a chitinous plate bearing a series of posterior teeth.

[1] In some species (*A. fluviatilis* and *A. culicifacies*) a pair are present on the thorax and on each of the abdominal segments except the last (James, p. 25).

(2) The *Culex* type, in which the spiracles are situated at the extremity of a siphon formed as a drawing out or prolongation of the dorsal region of the eighth abdominal segment. The two main longitudinal tracheal trunks are usually of a much greater diameter than in the *Anopheles* type, and apparently function to some extent as "air reservoirs." There are no palmate hairs, and the comb almost always consists of a variable number of scale-like spines.

(3) The *Sayomyia* type is, on the whole, the most modified among the Culicidae. The spiracles and respiratory tube are absent and the tracheal system is greatly reduced and mainly consists of two pairs of air vesicles which are situated respectively in the thorax and seventh abdominal segment.

Theobald (1905) recognises eight sub-families among the Culicidae viz. the Anophelinae, Megarhininae, Toxorhynchitinae, Culicinae, Joblotinae, Aedomyinae, Heptaphlebomyinae and Corethrinae.

The *Anophelinae* form a tolerably natural group and exhibit a great similarity among their larvae. The most obvious differences that are to be found are seen in the characters of the frontal, plumose, and palmate hairs, the antennae, the form of the comb and the labial plate.

The *Megarhininae* comprise the single genus *Megarhinus*. The larva is of the *Culex* type, but is remarkable in having the comb represented by a large chitinous plate and in the pecten being absent. It is said to possess a rudimentary spiracle on the last abdominal segment (Felt, 1905, p. 445). The larva of *Toxorhynchites* very closely resembles that of *Megarhinus* and it has been pointed out by Christophers (1906, p. 13) that they agree very closely in the shape of the head and clypeus, in having the brushes modified into "clasping organs" for seizing their prey and in the anal gills being reduced to stump-like papillae.

In *T. immisericors* (Walk.) according to Theobald (vol. iii. p. 118) and Green (1905, p. 159) the siphon is excessively short, and there are no anal gills and the larva rests very nearly parallel to the surface film.

The *Culicinae* exhibit a wide range of variation in their larval structure. The most striking and variable organ is the siphon which differs greatly in its length and general shape among various genera. Stephens and Christophers obtain what they term the "siphonic index" by dividing the length of the tube by its maximum breadth. This index is useful for comparative purposes and appears, on the whole, to

be very constant in certain genera. According to Christophers in *Stegomyia* the siphonic index varies between 1·6 and 2; in *Desvoidea* it is 1·7; in *Culex*, however, it varies very greatly being 4·3 in *Culex fatigans* Wied., while in *C. mimeticus* Noe, according to Theobald's figure (1903, vol. iii. p. 157) it is at least 14·5 on account of its extremely narrow diameter; in *Theobaldia* it is 6, while in *Taeniorhynchus* it is 13. In addition to the characters afforded by the siphon, numerous other structural variations occur, and some of which are proving of great utility in the specific determinations of the various larvae. The general form of the head, the characters of the antennae and the position of insertion of the antennal tuft, the form of the mouth-parts and especially of the labial plate are all of utility in the identification of species. Less useful characters are afforded by the variations in colour (very rarely) and in the form and arrangement of the cephalic and body setae. The anal gills present some degree of variation, being exceptionally long, according to Christophers, in a species of *Stegomyia* which mainly frequents the bottom of the water; in *Desvoidea* they are large and broad (Theobald, vol. iii. pl. XVI), and in most of the known larvae of this sub-family they are well developed, though in *Culex tigripes* Grand. et Charm. and *Acartomyia*, according to Theobald's figures, they appear to be wanting. A wide variation is exhibited in the pecten, or rows of teeth situated on the siphon and, similarly, much variability is seen in the group of scale-like structures which are collectively termed the comb, and situated on either side of the eighth abdominal segment.

Perhaps, the most divergent larvae of the genus *Culex* are those of *C. mimeticus* which, in addition to the features of the siphon already mentioned, are remarkable on account of the large curved pecten spines (Christophers, p. 10), and the cannibalistic species *C. concolor* Robin. Des. and *C. tigripes*. Both of the latter are highly modified, showing but little relationship with the *Culex* larvae and whose affinities are with *Megarhinus* and *Toxorhynchites*. Christophers suggests provisionally the enlargement of the sub-family Megarhininae so as to include, in addition to *Megarhinus*, *Toxorhynchites* (thus doing away with the sub-family this genus represents), *Mucidus*, *Psorophora* together with *Culex concolor* and *C. tigripes*, and possibly *Janthinosoma* and *Lutzia*. All these forms are specially modified for cannibalistic and carnivorous habits in general. Their brushes are transformed into clasping organs for seizing their prey, the mouth-parts are also specially modified and the shape of the head and clypeus is very characteristic.

The anal gills are either reduced to mere stumps or are completely absent, and the attitude of these larvae in relation to the surface film is much more horizontal than is usual among larvae of the Culicinae. The two species, *C. concolor* and *C. tigripes*, Christophers proposes to place in a new genus *Jamesia*, and provided the larval characters are corroborated by sufficiently important differences in the imago, this procedure is fully warranted. Furthermore, the remarkable larva of *C. mimeticus* suggests the possibility that a new genus may be desirable in this instance also.

It seems highly probable that, as our knowledge of the Culicidae becomes more extended, a rational classification of the family will have to be based on a combination of both larval and imaginal characters, and in no other family of Diptera do the larvae apparently exhibit such exceptionally well-defined features, among both genera and species, for this purpose. At present however, until the morphology and ethology of the larvae have been more critically studied and how far any particular modification may be correlated with a certain mode of life, it seems very nearly impossible to discriminate between those characters which have been developed, perhaps recently, by adaptation and those which are to be regarded as phylogenetic or morphological characters. Until this distinction is clearly recognised the application of larval characters for the purposes of classification loses much of its value. Dyar and Knab (1906) have recently contributed an important paper in which they classify the larvae of the New World mosquitoes independently of the imagines. It remains to be seen, however, how far this scheme will fit in with any system of classification founded on imaginal characters.

Of the *Joblotinae* our knowledge of the larval stage is based on a very brief description given by Theobald (1903, vol. iii. p. 334) of the young larva of *Joblotia niveipes* and the later account of Goeldi (1905, p. 120). It appears to be an extremely divergent form with stout blunt antennae and a pair of remarkable "frontal processes." The latter, however, seem to be really the maxillae. There is a short respiratory siphon of very unusual form, but there seem to be no lateral comb.

In the *Aedomyinae* the larval stages are very little known. In *Aedes fuscus* Osten-Sac. the larva is of the general *Culex* type and possesses four narrow lanceolate gills and a very short siphon (Dyar, 1902, p. 197). According to Felt (1904, p. 340) it so nearly resembles the larvae of *C. Sylvestris* Thed. and *C. impiger* Walk. that it

is difficult to separate them. The larva of *A. smithii* Coq. differs, however, so greatly from that of *A. fuscus* that the systematist if he were relying solely on larval characters would unhesitatingly place it in a separate genus. According to Dyar (1901, p. 178) it possesses but a single pair of anal gills which are broad and rounded at their apices; in the number of its gills it seems to be unique among Culicid larvae and Dyar and Knab regard the upper pair as having been aborted. The mandibles have but a single bristle or curved spine at the apex and furthermore the pecten is entirely wanting. From *A. fuscus* it further differs in the form of the thorax and siphon.

In *Uranotaenia* the larva closely resembles that of a typical *Culex* but the comb is remarkable in that it consists of eight simple spine-like processes attached to the posterior margin of a lateral plate of chitin, and recalls the comb plate of the Anophelinae (Dyar, 1901, p. 179; Felt, 1904, p. 343). The larva of *Deinocerites* is only known from very brief notes given by Theobald (1903, vol. iii., p. 280) and it is not possible to compare it with other forms. His account is also rather obscure, since he confuses the thorax partly with the first two abdominal segments.

The *Heptaphlebomyinae* include but a single genus whose larva is unknown.

In the *Corethrinae* the larval stages are known for all the described genera. That of *Sayomyia*[1] represents the extreme type of larval development met with in this sub-family. It is almost perfectly transparent, the extremities of the jaws and the pigmented eyes and air reservoirs being the only parts that catch the unaided eye. The fore part of the head is much prolonged, carrying the antennae at the extremity. The pharynx is modified into an eversible tube, and there are remarkable posterior hooks carried on the last abdominal segment. Respiration appears to be almost entirely cutaneous, though a greatly reduced and much modified tracheal system is present. It consists of a pair of longitudinal trunks extending through the greater part of the animal, but does not contain air, except in the thorax and the seventh abdominal segment. In these positions, however, there are situated a pair of pigmented air reservoirs. There are no traces of spiracles present. Situated on the ninth segment of the abdomen are two pairs of anal gills.

The larva of *Corethra*[2] forms a connecting link between that of

[1] *Sayomyia* Coquill. (*Corethra* Loew).

[2] *Corethra* Meigen (*Mochlonyx* Loew).

Sayomyia and a typical Culicid larva. The intermediate condition is especially well indicated in the tracheal system which, although it has undergone some amount of reduction, is much better developed than in *Sayomyia* and, moreover, spiracles are present. They are situated at the extremity of a short respiratory siphon arising from the eighth abdominal segment. *Corethra* resembles *Sayomyia* in the form of the antennae but the head is not prolonged anteriorly (Meinert, Tab. II). The larva of *C. Karnerensis* is remarkable in having the extremity of the last segment bordered by a whorl of numerous, short, recurved fleshy processes (Felt, 1904, p. 353).

The larvae of both *Eucorethra* and *Corethrella* resemble *Corethra* much more closely than *Sayomyia*. Both possess a well developed tracheal system resembling that of *Culex* and opening at the extremity of a short siphon. The antennae in *Eucorethra* resemble those of the two preceding genera while those of *Corethrella* are curiously folded back against the head (Felt).

Pelorempis is a recently discovered form and very imperfectly known. It resembles *Sayomyia* and *Corethra* in the elongated antennae which are provided with stout spines set at an angle with their long axes. It has the general shape of the head of *Corethra* but the maxillae rather resemble those of *Sayomyia*. In its respiratory system it comes closer to the Anophelinae than to any other of the Culicidae. There is no siphon and the spiracles are supported by a chitinous skeleton resembling very closely that of *Anopheles* (Johannsen, p. 404). *Pelorempis* seems, therefore, to be a synthetic type as regards its larval characters, since it exhibits features belonging to both Corethrinae and Anophelinae.

The relationships of the larvae of the Corethrinae appear on the whole to come nearest to the Culicinae. The fact that they are predaceous, feeding on larvae of their own and other species, and small Entomostraca, &c., might suggest the possibility of their having arisen from the Megarhininae (*sensu latiore* of Christophers) which are similarly carnivorous (and cannabalistic). Such a view, however, would not be tenable since, in the Corethrinae, the antennae are the organs which are specially modified for seizing the prey, while in the latter group, it is the feeding brushes that have become modified for the same purpose.

Rondani in 1856 appears to have been the earliest writer to separate the Corethrinae from the rest of the Culicidae and elevate them to family rank. More recently Coquillett and others have also

advocated this classification. Great stress has been laid on the reduced condition of the mouth-parts which are not adapted for piercing and, correlated with it, the feeding habits of the female. Additional characters have also been found in the neuration of the wings and the absence of scales from the latter. It is the belief of the author of this paper, however, that the Corethrinae are best retained as a sub-family of the Culicidae.

The reasons for this conclusion are as follows:—

The newly discovered genus *Pelorempis*, though agreeing with *Sayomyia* and *Corethra* in the form of the proboscis, resembles the Culicinae in possessing scales to the wings. The larva, moreover, as has already been pointed out, greatly differs from those of the remaining Corethrinae. The *Sayomyia* larva, it is true, is totally different from any other Culicid larva, but those of the remaining Corethrinae are clearly intermediate in the structure between that genus and the general *Culex* type of larva. It is, therefore, clear that the limits of the Corethrinae cannot at present be very well defined and, for that reason, it is better to retain the group as a sub-family only.

The resemblances between the larvae of the Anophelinae and the Culicinae, as regards their internal anatomy (the presence of cardiac coeca and five Malpighian tubes), and the great divergence between those groups and the genus *Sayomyia* (four Malpighian tubes and the absence of cardiac coeca), are sufficient to suggest that an anatomical study, of typical representatives of the different groups, may prove a valuable aid towards a rational classification of the family.

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abd. 5, 6, 8, 9.	Fifth, sixth, eighth and ninth abdominal segments.
abd. g. 1.	First abdominal nerve ganglion.
abd. g. 8+9.	Ganglion formed by the concrescence of those of the eight and ninth abdominal segments.
al. m.	Alary muscle.
a. m.	Adductor muscle of mandible.
a^1. m^1.	Abductor muscle of mandible.
a. n.	Antennal nerve.
ao.	Aorta.
ap.	Aperture of salivary duct.
at. d.	Imaginal bud of antenna.
at. m.	Antennal muscle.
b.	Brush.
b. m.	Inner retractor muscle of brush.
b^1. m^1.	Outer ,, ,, ,, ,,
c.	Cuticle.
c. c.	Lateral cellular cord.
c. com.	Cerebral commissure.
d.	Rudiment of generative duct.
d. tr.	Main longitudinal tracheal trunk.
d. v.	Dorsal vessel.
e.	Eye.
ep. m.	Epipharyngeal muscles.
f. b.	Fat-body.
f. n.	Frontal nerve.
f. pl.	Fan-like plate.
g.	Abdominal nerve ganglion.
g. d.	Imaginal buds of gonapophyses.
g. l.	Ganglionic layer.

h.	Hypodermis.
i. mus.	Inner vertical muscle of spiracular lobe.
l. d.	Imaginal bud of left middle leg.
l^2. d^1.	Imaginal bud of labium ; left of the paired elements (section somewhat oblique).
l. e.	Larval eye.
l. musc.	Longitudinal muscles of spiracular lobe.
l. pl.	Lateral plate of spiracular lobe.
l. t. m.	Longitudinal tergal muscles.
m.	Muscle attached to supporting collar of heart.
m. b.	Striated muscle bands (in section).
m. c.	Mother cells.
m. g.	Mid-gut.
m. n.	Median nerve.
m. pl.	Median plate of spiracular lobe.
m. s.	"Mesenchyme."
m. x.	Muscle of maxilla.
ner.	Lateral nerve.
o.	Ommatidia.
oen.	Oenocytes (smaller).
o. mus.	Outer vertical muscles of spiracular lobe.
o. n.	Optic nerve.
p.	Chitinous peg.
p. c.	Pericardial cells.
ph.	Pharynx.
ph. w.	Wall of pharynx.
pl.	Lateral toothed plate.
p. m.	Peritrophic membrane.
p. s.	Peripodial space.
r.	Rectum.
sc.	Basal supporting plate of spiracular skeleton.
s. col.	Supporting collar of heart (*anneau de soutien*).
s. col. p.	Posteriorly directed prolongations of the supporting collar.
s. d.	Median salivary duct.
s. g.	Sub-oesophageal ganglion.
sp.	Spiracles.
sup. g.	Brain.
t.	Tracheal trunk to anal gills of its side.
t. b.	Transverse chitinous band.
t^1. b^1.	Transverse intersegmental band.
t. f.	Larval "tail-fin."
t. f. m.	Muscles of larval "tail-fin."
t^1. f^1.	Terminal filament.
t. f. d.	Imaginal bud of pupal "tail-fin."
th. g. i.	Prothoracic ganglion.
t. pl.	Terminal plate of spiracular lobe.
tr.	Trachea.
w. d.	Imaginal bud of wing.
x - - - y	Direction of the transverse section Fig. 2.

DESCRIPTION OF THE PLATES.

PLATE IX.

Fig. 1. Dorsal aspect of the head of a larva of *Anopheles maculipennis* showing certain of the muscles.

Fig. 2. Transverse section through the head across the line x—y in the preceding figure.

Fig. 3. Typical transverse section through the abdomen and passing through one of the ventral nerve ganglia.

Fig. 4. Lateral view of the spiracles and their supporting skeleton.

Fig. 5. Transverse section through the eighth abdominal segment together with the spiracular lobe. (Reconstruction from several sections.)

Fig. 6. Transverse section through the ninth abdominal segment showing the muscles of the "tail-fin." (Reconstruction from several sections.)

Fig. 7. Longitudinal and horizontal section through the spiracular lobe passing some distance below the level of the spiracles.

Fig. 8. Do. passing just beneath the spiracles.

Fig. 9. Transverse section through the heart and associated structures; 6th abdominal segment.

Fig. 10. Dorsal aspect of a portion of the heart showing alary muscles and pericardial cells; 6th abdominal segment.

Fig. 11. Rudiment of the male gonad from larva measuring 6·5 mm. long.

Fig. 12. Rudiment of the female gonad from a larva measuring 6·75 mm. long. The numbers in this and the preceding figure represent the respective segments in which the organ lies, and the horizontal lines indicate the boundaries between those segments.

Fig. 13. Transverse sections through three separate regions of the male gonad of a larva about 6 mm. long: (*a*) through the base of the terminal filament; (*b*) through the middle of the gonad; (*c*) through the developing duct.

Fig. 14. Outline figure of the rudiment of the male gonad in a larva 2·3 mm. in length.

Fig. 15. Transverse section through the heart and associated cellular cords; anterior part of the thorax.

Fig. 16. Do. through the heart and posterior prolongations of the supporting collar; commencement of oesophagus.

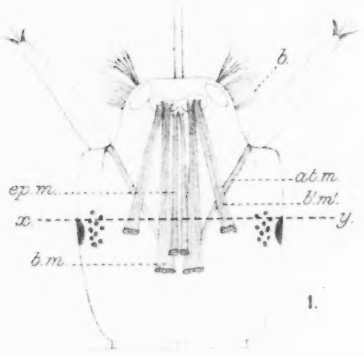
Fig. 17. Do. through the middle of the supporting collar together with the heart.

Fig. 18. Do. through the cerebral commissure and the anterior termination of the heart.

Fig. 19. Section of imaginal buds of wing and second leg of left side at an early stage in development.

Fig. 20. Section through imaginal antennal bud and passing through the opening of the primitive invagination.

Fig. 21. Longitudinal and vertical section passing through the cerebral commissure together with the terminal portion of the heart and the associated supporting collar.



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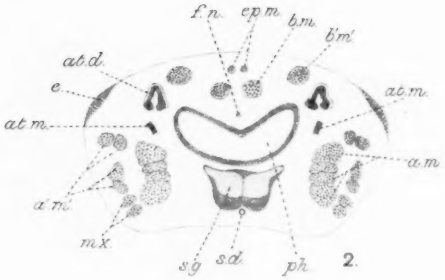

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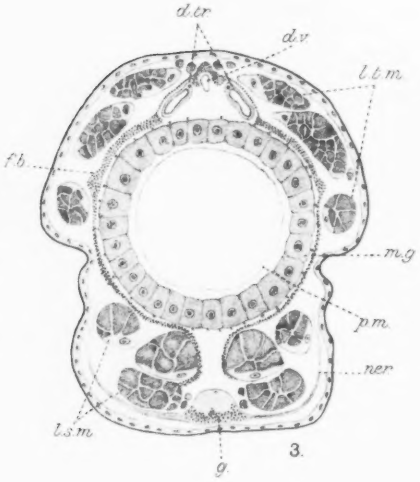

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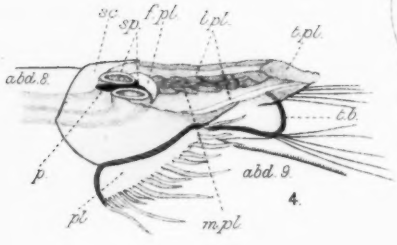

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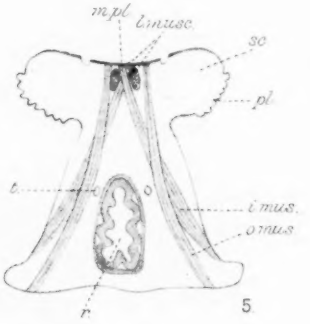

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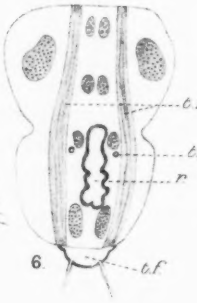

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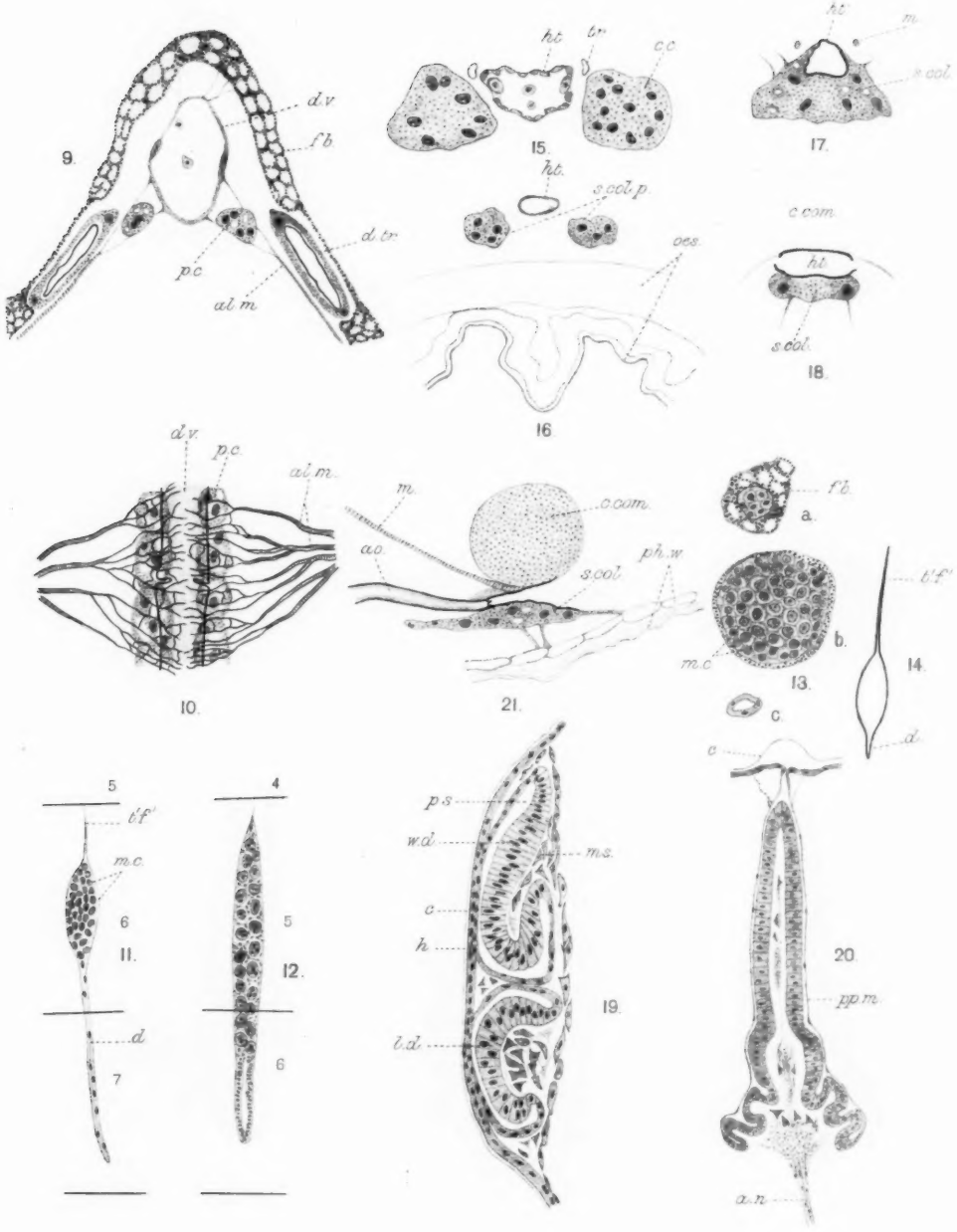
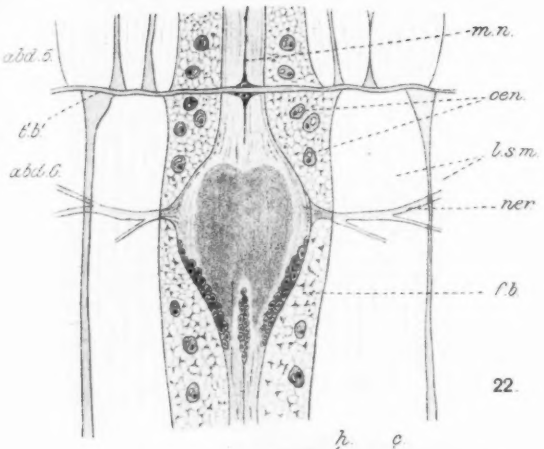
PLATE IX



E. Wilson, Cambridge.



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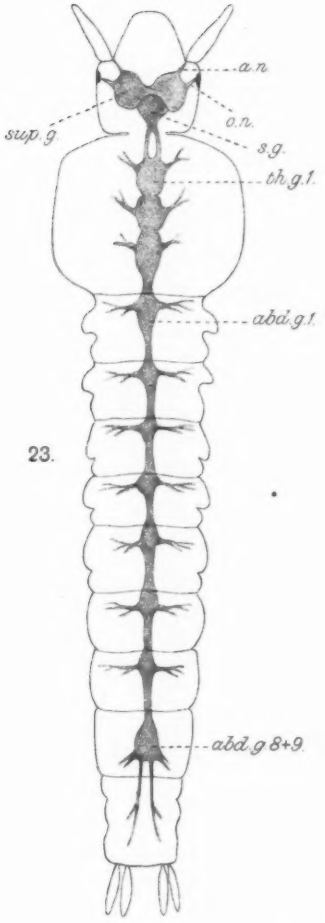

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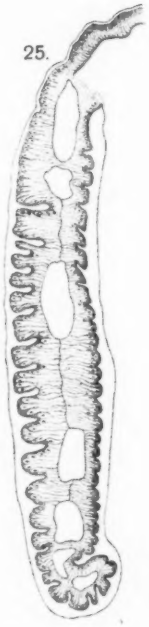

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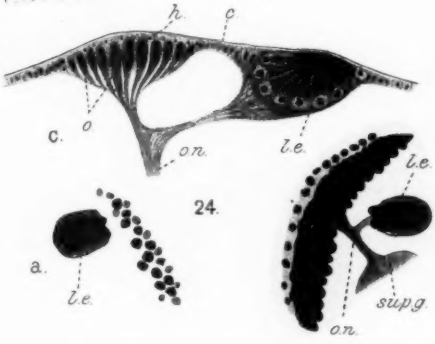

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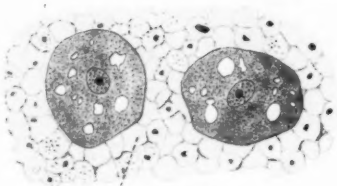

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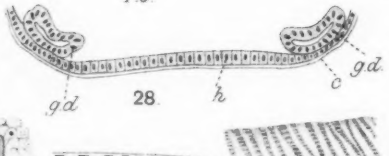

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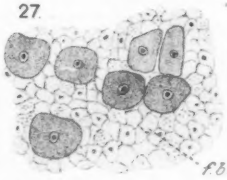

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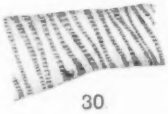

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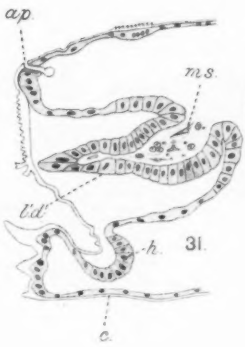

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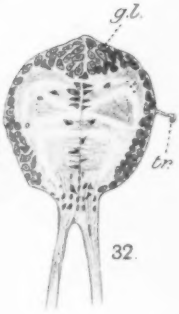

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PLATE X.

Fig. 22. Figure of the ventral region of the 6th abdominal segment showing its nerve ganglion, the fat-body and oenocytes. Longitudinal and horizontal section.
Fig. 23. Nervous system of a larva about half-grown.
Fig. 24. Larval and developing imaginal eyes: (*a*) from a larva 4·25 mm. long; (*b*) from a larva 7 mm. long; (*c*) do. viewed in section. Figs. *a* and *b* are from larvae cleared and mounted whole in Canada balsam.
Fig. 25. Section through left imaginal wing bud: fully grown larva.
Fig. 26. A pair of the larger oenocytes: 5th abdominal segment.
Fig. 27. Group of the smaller oenocytes.
Fig. 28. Section through ventral integument and imaginal buds of gonapophyses.
Fig. 29. Longitudinal section through the walls of the heart: 8th abdominal segment.
Fig. 30. Portion of the heart showing striated fibres: 8th abdominal segment.
Fig. 31. Longitudinal and vertical section through labial plate and imaginal labial bud (left rudiment).
Fig. 32. Longitudinal and horizontal section through the sub-oesophageal ganglion.

THE MODE OF MULTIPLICATION OF *PIROPLASMA BOVIS*, *P. PITHECI* IN THE CIRCULATING BLOOD COMPARED WITH THAT OF *P. CANIS*, WITH NOTES ON OTHER SPECIES OF *PIROPLASMA*.

Plate XI and Diagrams I—IV.

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University Lecturer in Hygiene, Cambridge.

In our last paper dealing with *Piroplasma canis* (*Journal of Hygiene*, April 1907, VII. pp. 232—272), we described the usual mode of multiplication in the circulating blood, as ascertained by a long series of observations on the *living parasite*. In summarising these observations we say (p. 250):—

"*Piroplasma canis* has a free and an intracorpuscular stage in the blood of the dog, and it is during the latter stage that multiplication occurs. This asexual multiplication takes place in one of the following ways.

(1) A free pyriform parasite which has just left a blood corpuscle enters a normal corpuscle and assumes a round form, remaining quiescent for a time. The round body then grows and, after passing through an actively amoeboid stage, again becomes rounded. Two symmetrical processes are then protruded, which rapidly enlarge at the expense of the body of the parasite. Each of these processes gives rise to a mature pyriform parasite, which remains for a time joined to its fellow by a thin strand of protoplasm. On the rupture of the containing corpuscle these pyriform parasites become free and enter other corpuscles.

(2) Occasionally by the protrusion of four processes four mature pyriform parasites are formed from a single amoeboid form.

(3) Sometimes a young rounded intracorpuscular parasite divides by simple division and gives rise to two amoeboid parasites, which grow and divide by the protrusion of symmetrical processes each into two pyriform parasites, thus giving rise to four mature parasites within the corpuscle. Sometimes the two amoeboid parasites undergo the processes of division simultaneously, but not infrequently one is considerably in advance of the other.

(4) It is possible that occasionally a red blood corpuscle is invaded either simultaneously or at different times by two pyriform parasites, each of which undergoes the changes described above.

(5) Several pyriform bodies within a single corpuscle are produced by the division in the manner described of one or more amoeboid parasites.

All parasites which have not reached the mature stage, when the containing corpuscle ruptures, rapidly degenerate and die in the plasma. The same is true of mature pyriform parasites, which do not, after becoming free, soon enter other corpuscles."

Aided by the knowledge thus gained we were able, by the examination of stained films, to figure the nuclear (chromatin) changes which accompany each phase of division (1907, Plate II). A schematic representation of the mode of multiplication was given on p. 258, and is reproduced in this paper (p. 136). We now figure some of the more important stages by means of photomicrographs (see Plate XI).

Christophers (1907, p. 17), who at the time of writing had apparently not seen the paper just quoted, and who seems to have formed his conclusions on the study of stained specimens only, thinks that besides the method of division we have described the formation of the pear-shaped forms by fission occurs in two other distinct ways.

"(1) In the case of certain large parasites, characteristic of early invasion and infection in young dogs, the division takes place in the manner described by Nocard and Motas. After the chromatin has divided a dark line forms across the parasite in the centre of which a cleft is later seen. This stage gives rise to two closely approximated bodies. (2) A method of division, almost if not quite as common, takes place when a parasite is stretched across a cell. The long oval becomes constricted in the centre, and the two ends passing away from one another become two pear-shaped bodies."

Although we have made very prolonged and careful studies at all

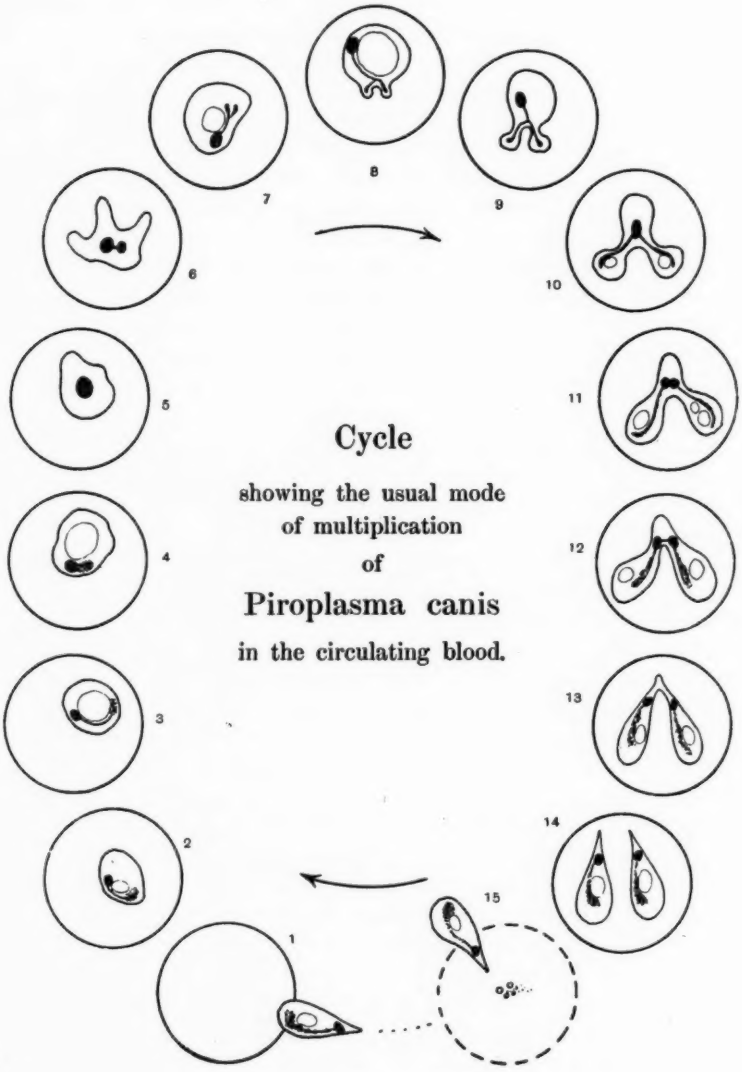

Diagram I. (Reprinted from *Journ. of Hygiene*, vol. VII. p. 258.)

stages of infection by inoculation on the large and elongated forms seen in the living blood we have never observed the formation of pyriform bodies by either of these methods, and cannot accept conclusions based

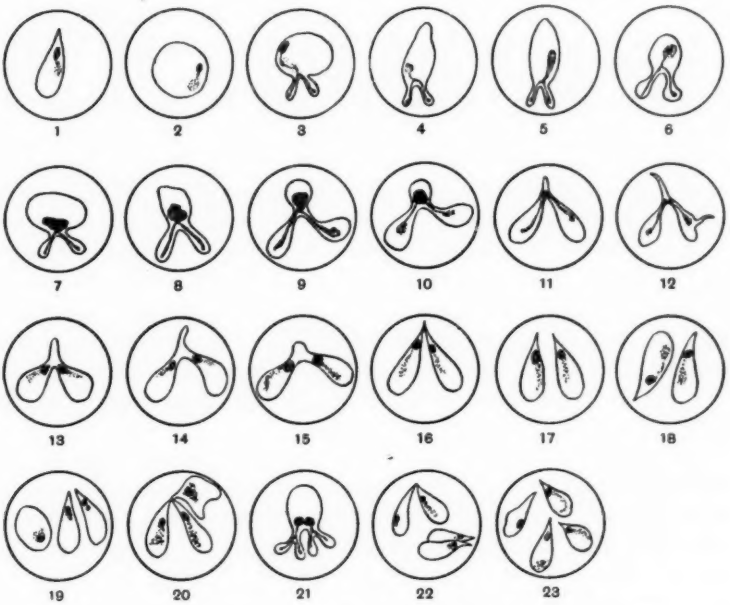

Diagram II. (*Piroplasma bovis.*)

Explanation of Diagram II showing multiplication forms of P. bovis compared with those of P. canis (*Diagram I*).

Stages figured in Diagram II (*P. bovis*)	... which correspond to ...	Stages figured in Diagram I (*P. canis*)
Figs. 1, 2, 3, 4, 5, 6	... ...	1, 2, 8, 8, 9, 9.
,, 7, 8, 9 ...	... ...	Stages between 9 and 10.
,, 10, 11 ...	... ...	10.
,, 12	... ...	11.
,, 13, 14, 15 ...	... ...	Stages between 12 and 13.
,, 16	... ...	13.
,, 17, 18 ...	... ...	14.

The remaining stages correspond to other Diagrams as follows:

Figs. 19, 20	1906, Diagram 6, Figs. 1, 2 (p. 596).
,, 21	1907, Diagram 27, Fig. 3 (p. 240).
,, 22, 23	1906, Diagram 6, Figs. 7—16.

on the study of stained specimens against the evidence derived from observations on living parasites.

In the paper quoted we have shown that single parasites do undoubtedly divide by simple division (Diagram 28, p. 240) but in this case give rise to two amoeboid forms, each of which may proceed to the formation of pyriform bodies (Diagrams 30, 31 and 36, pp. 241, 242, 262) in the manner we have described.

Stained specimens of such preliminary divisions and apparent divisions (Diagram 29, p. 241) may have influenced Christophers in his conclusions.

In stained preparations *Piroplasma canis* can be distinguished from other intracorpuscular blood parasites by the occurrence of intracorpuscular pyriform bodies, which represent the mature form of the parasite in the blood. These pyriform bodies are most commonly present in the corpuscles in pairs, though multiples of two, four, eight and sixteen, are not uncommon. These forms generally show two chromatin masses, a dense and compact mass usually situated near the pointed end and a secondary mass of loose chromatin[1] which may be arranged in various ways, extending towards the blunt end.

Dividing forms, fixed in the later stages of division, especially the trilobed forms which are of frequent occurrence in suitable films, are yet more characteristic. By a careful study of stained films we have been able to establish the fact that two other species of *Piroplasma*, *P. bovis* and *P. pitheci*, divide in the same manner as *P. canis*, and show the same chromatin masses.

Piroplasma bovis.

After examining a considerable number of blood films, each of which contained only a few parasites with negative results as regards the finding of dividing forms, we obtained a slide prepared from a cow suffering from a high degree of infection, through the kindness of Mr J. R. Jackson, M.R.C.V.S., of the Board of Agriculture and Fisheries. In parts of this film as many as 30 infected corpuscles could be counted in a single field. Double but especially single pyriform bodies containing both dense and loose chromatin were common. The film was

[1] This secondary mass of chromatin was first described by us (*Journal of Hygiene*, x. 1906, p. 590) and has since been noticed by Christophers (1907, p. 22) in *P. canis*, who says "the loosely packed area described by Nuttall and Graham-Smith is almost invariably present," and by Fantham (1907, p. 303) in *P. bovis*.

taken at a period of very active multiplication, for typical dividing forms were found in almost every field[1].

We have figured a selected series in Diagram II, which may be compared with our schematic figure of the usual mode of multiplication in *P. canis* which we have reproduced (Diagram I).

P. pitheci, P. H. Ross, 1905.

This parasite was discovered by Dr Philip H. Ross in the blood and internal organs of a species of *Cercopithecus*, and was described by him in the *Journal of Hygiene*, I. 1905, v. pp. 18—23. As he did not illustrate the parasite the true nature of the organism still remained in doubt.

Through the kindness of Dr Ross we have had the opportunity of studying his slides. One blood film contained only a single parasite, but another contained over one hundred. A smear preparation from the liver contained large numbers of parasites but was not as well fixed or stained as the blood preparations. All these preparations were systematically examined and the parasites sketched[2].

These observations have convinced us that the parasite described by Ross is a true *Piroplasma*, and multiplies in the same manner as *P. canis* and *P. bovis*.

The number of parasites contained in infected corpuscles varied from 1 to 16.

	Number of parasites per infected corpuscle	
	In blood films	In liver smear
1	Common	Common.
2	,,	,,
3	One found	None found.
4	Found 7 times	Fairly common.
5	Not found	Not found.
6	,,	,,
8	Found 6 times	Fairly common.
16	Not found	Two found.

The prevalence of the paired forms and the occurrence of the usual multiples 2, 4, 8, 16, accord with what we have observed in *P. canis*.

[1] Of 500 consecutive infected corpuscles examined 55 % contained single parasites, 34 % double parasites and 11 % contained parasites in the typical process of division. We would particularly emphasize the fact that numerous dividing forms are only to be occasionally met with, consequently negative results, on the examination of single films, or even a series of films from a single animal, cannot be accepted as evidence against this being the usual mode of division.

[2] Our thanks are due to Mr C. Strickland, who searched through the second blood preparation with great care.

The series of figures included in Diagrams III and IV are selected from the large number of sketches which we made.

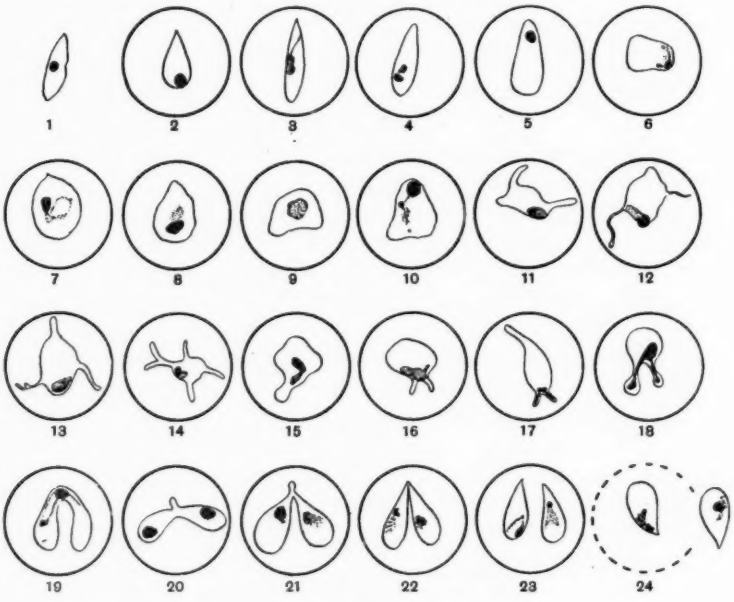

Diagram III. (*Piroplasma pitheci.*)

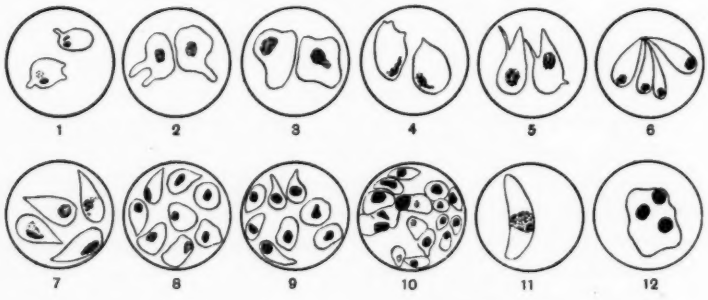

Diagram IV. (*Piroplasma pitheci.*)

We have described and figured similar stages in *P. canis* (see Diagram I, p. 136; also *Journal of Hygiene*, x. 1906, and iv. 1907.

Piroplasma muris, Fantham, 1906.

Through the courtesy of Mr H. B. Fantham we have been able to examine one of his films prepared from the blood of a rat infected with *P. muris*. This preparation contained pyriform intracorpuscular parasites, singly and in pairs. In the plate accompanying his paper corpuscles infected with four and six parasites are shown. The secondary mass of loose chromatin has not been observed and no typical dividing forms have been found. The latter fact may partially be accounted for by the low degree of infection and the lack of material. Further the fact that the disease is a chronic one renders the presence of many dividing forms at one time improbable.

Piroplasma equi and *P. ovis*.

We have as yet been unable to secure satisfactory specimens of these organisms, and none of the papers which have been published have contained figures or records of dividing forms such as we have described in *P. canis*.

Note on *Achromaticus vesperuginis*, Dionisi 1898.

This parasite is regarded as a *Piroplasma* by Sambon[1], 1908, in Manson's *Tropical Diseases* (4th edition, p. 841), but we fail to see sufficient reason for this assumption. It is true that both Dionisi and Gonder figure a few pyriform parasites amongst the many they illustrate, and we have found similar forms in films Dionisi gave to one of us in 1899.

In only one other respect does this organism resemble a typical *Piroplasma*, namely in the occurrence of four pyriform parasites within the corpuscles. Further investigations are required before its position can be settled[2].

SUMMARY.

In stained preparations *Piroplasma canis*, *P. bovis* and *P. pitheci* may be distinguished from other intracorpuscular parasites by the presence of intracorpuscular pyriform bodies, usually occurring in pairs and less commonly in fours, eights and sixteens. These pyriform bodies show a dense mass of chromatin near the pointed end and a loose mass, often connected with the dense mass, situated towards the blunt end. In suitable preparations peculiar dividing forms, most typically represented by trilobed forms or more or less pyriform bodies joined to a single smaller rounded or elongated mass of protoplasm, may be seen.

[1] We regret to note several inaccurate statements which occur in Sambon's section dealing with the genus *Piroplasma*.

[2] Gonder (1906, pl. IV) retains the name *Achromaticus* believing it to be intermediate between human malarial parasites and *Piroplasma*.

In the absence of observations on the *living parasite* we consider that these points may be taken as characteristic of the genus *Piroplasma*.

In spite of the fact that dividing forms have not yet been found and that the secondary mass of chromatin has not yet been observed *P. muris* may perhaps be included in this genus.

Piroplasma quadrigemium, an intracorpuscular parasite recently observed by Nicolle (XI. 1907) in a small North African rodent (*Ctenodactylus gundi*), apparently divides in a totally different manner, and shows a peculiar disposition of the chromatin. No loose chromatin has been observed. Until further observations have been made this parasite cannot be included among the true *Piroplasma*.

Further observations are needed before the position of the other so-called *Piroplasma* can be determined.

The expenditure entailed in the prosecution of our investigations on piroplasmosis has been largely defrayed by grants from the Government Grants Committee of the Royal Society.

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EXPLANATION OF PLATE XI.

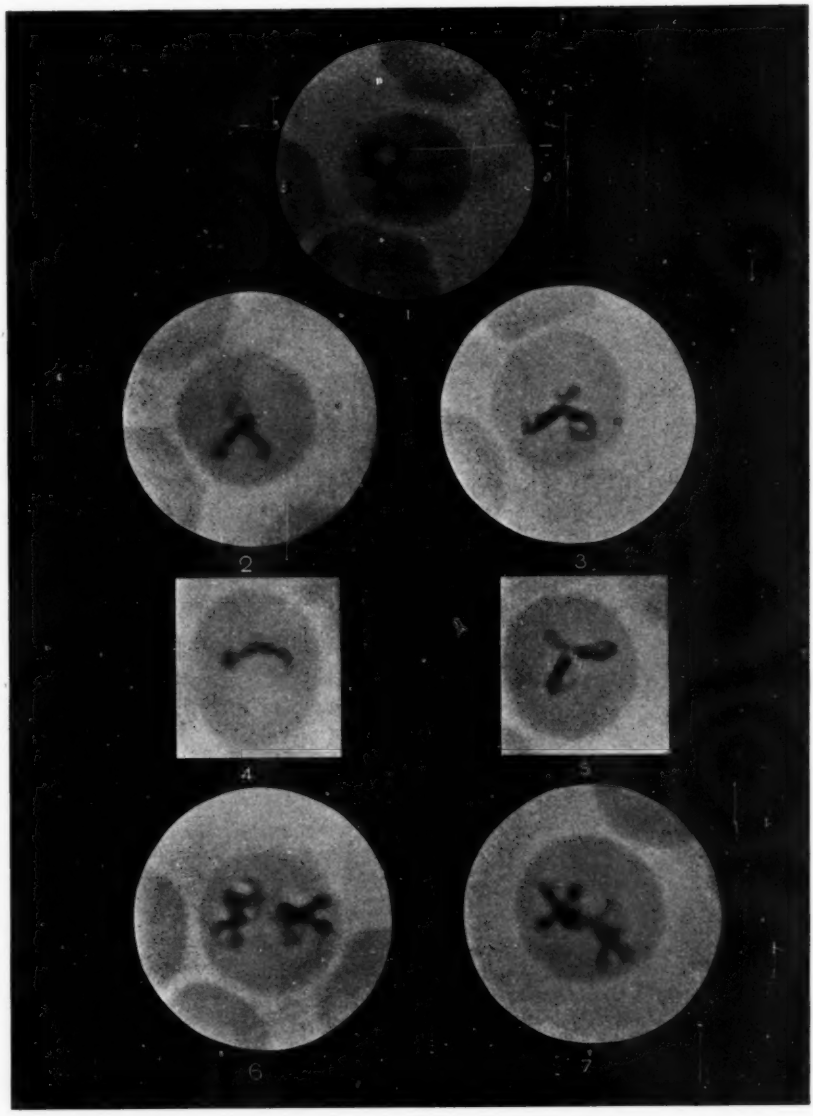
Multiplication of *P. canis*. Examples taken from a single blood-film. Photomicrographs, × 3000.

Figs. 1, 2 correspond to Figs. 8—10 in Diagram I (p. 136).

Figs. 3, 4 correspond to Fig. 12.

Fig. 5 corresponds to Fig. 13.

Figs. 6, 7 correspond to Figs. 7, 8 in Diagram 36 (1907, p. 262), and each illustrate the simultaneous division of two parasites in a corpuscle.



PIROPLASMA CANIS

Illustrating the process of multiplication.

NOTE ON THE BEHAVIOUR OF *SPIROCHAETAE* IN *ACANTHIA LECTULARIA*.

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A POPULAR belief prevails in Russia that the bug *Acanthia lectularia* plays a part in the spread of relapsing fever. Flügge (1891) however appears to have been the first scientific writer to suppose that vermin might spread the disease. This view was also held by Tictin (1897) who considered that man may become infected by (*a*) being bitten by bugs which had previously fed on blood containing *Spirochaeta obermeieri*, or (*b*) by his crushing such bugs and infecting himself with the spirochaetes through lesions in the skin induced by scratching. Tictin injected the gut-contents of infected bugs which had recently fed on a relapsing fever patient into monkeys and infected them. When the bugs were crushed 48 hours after feeding on infected blood their contents did not produce the disease in monkeys.

Karlinski (1902) observed *Spirochaeta obermeieri* in 120 bugs examined during the prevalence of relapsing fever in Bosnia. The bugs were collected in houses where cases of relapsing fever occurred. The spirochaetes retained their motility in the bugs for 30 days after the initial feeding, but later they appeared to be degenerating. (The examination of fleas and lice gave negative results.) Schaudinn, not long before his death, informed me that he had observed an even longer persistence of the spirochaetes in bugs. Christy (1902), working in Bombay, placed bugs in the beds of relapsing fever patients and afterwards allowed twelve of the bugs to bite him, one bug biting him each day on twelve successive days. He did not however acquire relapsing fever. Breinl, Kinghorn and Todd (1906, p. 113) do not state that they made

observations on the behaviour of spirochaetae in bugs. They however published the results of ten experiments (conducted in Liverpool) on monkeys which they sought to infect with spirochaetes through the agency of *Acanthia lectularia*. Six of the ten experiments were carried out with *Spirochaeta duttoni* alone, whilst four were carried out with bugs infected partly with *Sp. obermeieri* (American strain) and partly with *Sp. duttoni*. It is not quite clear from their paper how many bugs were infected with the latter spirochaete alone. Considering the experiments collectively, the authors named allowed 10 monkeys to be bitten by 2210 bugs which had previously fed on infected animals. The bugs sucked infected blood at different stages of the disease and in turn bit fresh uninfected monkeys at variable periods of time (2 to 3 or 10 to 15 days) after being infected. A few of the bugs were kept at a low temperature (5·5° C.) after sucking infected blood, but the majority were maintained at 20—21° C. All their results were negative.

Klodnitzky (1908, p. 127) in Astrachan, allowed bugs to bite relapsing fever patients and also collected supposedly infected bugs in hospital wards. He examined the contents of about 30 bugs and detected isolated spirochaetes in them for 3—5 days after they had fed, and subsequently he observed what he supposed was a great multiplication of the spirochaetes in the bugs. His description and the two photomicrographs which illustrate his paper leave no doubt in my mind but that he took the spermatozoa of the bug for spirochaetes.

From the foregoing it will be seen that we still lack evidence proving that *Sp. obermeieri* is conveyed by bugs. The negative results obtained by Breinl, Kinghorn and Todd do not however exclude the possibility of bugs being the carriers of relapsing fever, and certainly the results obtained with *Sp. duttoni* do not weigh as evidence against bugs being the carriers of *Sp. obermeieri*. There is no evidence to support the hypothesis of Dönitz (1907, p. 18) that European relapsing fever is conveyed by ticks. The observations by Karlinski and Schaudinn above cited indicate that further work on the subject is still required.

Failing *Spirochaeta obermeieri* it appeared to me desirable to study the behaviour of *Sp. duttoni* in *Acanthia lectularia* and with this object the following experiments were carried out.

Acanthia lectularia, received from London, were placed at 16° C. Hungry specimens were chosen and fed on mice in whose blood *Sp. duttoni* was present in large numbers. At the time stated in the first column, in minutes or hours, a bug (or two) was dissected and the intestinal contents examined fresh and stained by Giemsa's method (dry films).

Experiment I, at 12° C.

Bugs fed on	Time when bug was examined after feeding	Appearances of spirochaetes in gut-contents: Fresh	Stained
Series A 30. iv. 1907	10, 15, 15 mins.	Many, very active	Rigid spirals, few wormlike spirochaetes, all stain well incl. red blood corpuscles.
	½, 1, 1½ hrs.	Ditto, some spir. sluggish	Ditto.
	21, 27 ,,	All fairly active	Ditto.
	48, 74½ ,,	Fairly active, some sluggish	Ditto, but some r. b. c. stain faintly.
	95 ,,	All fairly active	Ditto.
Series B	100, 117 ,,	Sluggish	Ditto.
27. v. 1907	139 ,,	Saw only one moving: sluggish	Ditto.
	164, 190 ,,	Motionless	Ditto.
	210, 234 ,,	,,	Ditto, corpuscles few and vanishing.
	254, 278 ,,	,,	Ditto.

Experiment II, at 14° C.

3. v. 1907	122 hrs.	Very slight motion, spir. tangled	Many spirals, few wormlike, stain well; r. b. c. traces?
	144 ,,	Slight motion?	Ditto.
	167, 198, 213 hrs.	Motionless	No r. b. c. seen, spir. stain well.

Experiment III, at 16° C.

2. iv. 1907	10 mins.	Many, very active	Many, all wormlike.
	3½, 6½ hrs.	Fairly active	Few wormlike, mostly spirals.
	19 ,,	Active, in bunches	Mostly spirals, some thick, beaded or irregular; r. b. c. stain well.
	28 ,,	Fairly active, some motionless	Ditto, many spir. appear thick.
	42 ,,	Slight motion	Spirals stain faintly, r. b. c. vanishing.
	72 ,,	Not examined	No. spir. found, no r. b. c.
	92, 117 ,,	,, ,,	Fairly numerous spir., stain well.
	139 ,,	Few, motionless	Ditto, but stain faintly.

Experiment IV, at 20° C.

2. iv. 1907	3½ hrs.	Fairly active	Spirals and r. b. c. both normal.
	6 ,,	Fairly active, some motionless	Spirals, some beaded and irregular, r. b. c. vanishing.
	8½ ,,	Motionless	Spirals, dividing forms, beaded and irregular forms, very few r. b. c.
	22½, 31 ,,	,,	Mostly faint-staining, often fragmented, few or no r. b. c.
	46, 72 ,,	,,	Ditto, but no r. b. c.
	92 ,,	,,	Ditto, but very few pale spir. found.
	119 ,,	Very slight motion?	Well stained.
	139 to 331 hrs.	Not examined fresh	None found (series of 8 bugs examined).

Experiment V (a), at 24° C.

Bugs fed on	Time when bug was examined after feeding	Appearances of spirochaetes in gut-contents	
		Fresh	Stained
2. iv. 1907	3½ hrs.	Most spir. fairly active, some sluggish	Mostly spirals, stain well, a few beaded and dividing; r. b. c. vanishing.
	6 ,,	Very slight intermittent motion, some motionless	Many rigid spirals, a few pale-staining, others with one half thick and fragmented chromatin; r. b. c. vanishing.
	8 ,,	Motionless, some short, others with globular swelling.	Ditto.
	22 ,,	Motionless	Many, some normal, others slender or pale and fragmented, no r. b. c.
	31 ,,	None found	Ditto.
	46 ,,	,, ,,	Only 2 found, faintly stained, no r. b. c.
	72 ,,	Not examined	One bunch of spir. seen, fairly stained.
	96 ,,	,, ,,	None found.

Experiment V (b), at 24° C.

27. v. 1907	6, 8, 24, 48, 96 hrs.	Practically same result as Exp. V: motionless after 8 hrs., no spir. after 48 hrs.	6 hrs., spiral and wormlike forms, these and r. b. c. stain well; 8—24 hrs. ditto, r. b. c. vanishing; 48—96 hrs., none found, no r. b. c.

Summary of the foregoing experiments.

It will be seen from the foregoing protocols of the experiments that the spirochaetes still showed slight *movement* after

139 hours at 12° C. and were fairly active after 95 hours.
122 ,, 14° C.
42 ,, 16° C. and were fairly active after 28 hours.
6 ,, 20° C.
6 ,, 24° C. and were fairly active after 3½ hours.

In *stained films* the spirochaetes mostly appeared normal up to

278 hours at 12° C.
213 ,, 14° C.
19 ,, 16° C. though some were thick and beaded or irregular.
6 ,, 20° C. ,, ,, ,, ,,
3½ ,, 24° C. ,, ,, ,, ,,

No traces of spirochaetes could be detected in stained films after

139 hours at 20° C.
96 hours at 24° C.

Some *dividing forms* were seen after

8½ hours at 20° C.
3½ ,, 24° C.

The red blood corpuscles could still be discerned after

278 hours at 12° C.
?122 ,, 14° C.
42 ,, 16° C.
31 ,, 20° C.
8—24 ,, 24° C.

In no instance could the spirochaetes be found in the coelomic fluid of the bugs although they were searched for in about $\frac{2}{3}$ of the bugs tabulated in Experiments I—V.

Experiment VI.

This consisted in the *inoculation of mice* with the gut-contents of bugs maintained at a temperature of 12° C. after they had fed to repletion on the blood of a mouse suffering from infection with *Sp. duttoni*. The bugs fed at the height of the infection. The gut-contents of five bugs were used for the inoculation of each mouse, mixed with a drop of salt solution immediately before subcutaneous injection.

	Time when gut-contents of bugs were injected after bugs had fed on spirochaete blood	Time after inoculation when mouse showed first spirochaetes in its blood (examined every day)	Time after inoculation when the mouse died
Mouse I	21 hours	24 hours	7 days
,, II	51 ,,	48 ,,	19 ,,
,, III	95 ,,	168 ,,	28 ,,
,, IV	100 ,,	168 ,,	Recovered
,, V	120 ,,	72 ,,	23 days

Under ordinary conditions mice inoculated with *Sp. duttoni* show parasites in their blood after 14—20 hrs. They usually die on the 4th to 7th day. The sojourn in the bug for 21 hrs. at 12° C. does not seem to exert any influence on the spirochaetes, but after 51 hours there is a distinct delay with regard to the time when they appear in the blood of the mice and also with regard to the time when the mice die. This experiment establishes the fact that the spirochaetes survive for 5 days in the bug. The delay in their appearance in the blood of the mice may be due to the spirochaetes being present in reduced numbers or possibly to their having become attenuated. Judging from the results of Experiments I—VI it is probable that the spirochaetes may be infective after a still longer sojourn in the bug at a temperature of 12° C.

Conclusions (I).

The foregoing experiments show that *Spirochaeta duttoni* retains its virulence after a sojourn of 5 or more days in the intestine of *Acanthia lectularia* when the insect is maintained at a temperature of 12° C. Living (motile) spirochaetes were observed in the gut-contents of the bugs up to 6 days at 12°C. but only up to 6 hours at 20—24°C. The observations made at various temperatures appear to indicate that the spirochaetes are simply digested by the bug and that their more rapid disappearance from the insect's gut at higher temperatures is dependent upon the insect's digesting its food more rapidly when kept warm. The disintegration of the ingested red blood corpuscles in the insect's gut runs parallel to that of the spirochaetes, though the latter can be found in stained specimens long after the corpuscles have disappeared and the spirochaetes themselves have ceased to give any evidence of life.

Typical longitudinally dividing forms were encountered in stained preparation made from the contents of the bug's intestine 3½ to 8½ hrs. after the bug had fed on spirochaete blood, when the insects were maintained at 24° and 20° C. respectively.

The rapid death of the spirochaetes in bugs maintained at 20—24° C. may account for the negative results of the infection experiments carried out by Breinl, Kinghorn and Todd. Although the bug is not the true host of *Sp. duttoni* it is conceivable that it may serve as a mechanical carrier of the parasite. It has been proved that different species of Trypanosomes may be transferred by means of the bites of blood-sucking flies (*Glossina, Stomoxys*[1]) and that the plague bacillus may be transferred by means of *Acanthia lectularia*[2] when only a short interval (some minutes or usually less than 24 hours) elapses between the successive bites of the insect. Such experiments still require to be carried out with the bug and pathogenic spirochaetes.

Incidentally I may add that prior to accidentally losing the strain of *Sp. duttoni* it was maintained with undiminished virulence by passage through 100 mice during the course of one year.

I am indebted to Dr Levaditi of the Pasteur Institute for the strain of *Sp. duttoni* (R. Koch's) with which these experiments were carried

[1] See Nuttall (1907), for a summary of this subject.

[2] See Verjbitski (1908) in the references which follow.

out. Finally I should like to state that my laboratory assistant, Mr B. G. Clark, has been of material assistance to me in carrying out the foregoing investigation.

Note on Experiments with Spirochaeta obermeieri.

Since the foregoing pages went to press I have, through the courtesy of Geheimrath C. Fraenkel of Halle, succeeded in obtaining a strain of the true *Sp. obermeieri*, which had been brought from Russia. Although my experiments with this spirochaete have not been concluded, the results I have obtained appear worth recording, more particularly because a positive infection experiment with *Acanthia lectularia* was obtained.

Observations on the behaviour of Spirochaeta obermeieri in the body of Acanthia lectularia.

Experiment I, at 12° C.

Bugs fed on	Time when bug was examined after feeding	Appearance of spirochaetes in gut contents: Fresh	Stained
27. IV. 1908	6 hrs	Very active	Many rigid spirals, r. b. c. all staining well.
	22 ,,	Fairly active	Ditto, also a few worm-like.
	27 ,,	Sluggish	Ditto.
	47 ,,	Almost motionless	Ditto.
	71 ,,	? Motionless	Ditto.
	96 ,,	Motionless	Ditto, but r. b. c. begin to vanish.
	120 ,,	None found	None found, r. b. c. gone.

Note: One spirochaete found in coelomic fluid taken from leg after 6 hrs., the examination in all other cases proved negative.

Experiment II., at 25° C.

27. IV. 1908	3 hrs	Some very active others slow	Many rigid spirals, a few worm-like, r. b. c. all staining well.
	20, 27, 45 hrs	All motionless	Ditto, but r. b. c. fading.
	72 hrs	No spir. found	No spir., no r. b. c. found.

Note: No spirochaetes found in coelomic fluid from leg at any time.

Summary of foregoing experiment.

It will be seen from the foregoing protocols that the spirochaetes still showed *movement* after

27—47 hours at 12° C. but were motionless after 96 hours.
3 ,, 25° C. ,, ,, 20 ,,

In *stained films* the majority of the spirochaetes appeared normal up to

96 hours at 12° C., they disappeared after 120 hours.
45 ,, 25° C. ,, ,, 72 ,,

Some *dividing forms* were detected after 22 hrs. at 12° C.
The *red blood corpuscles* could be discerned after

96 hours at 12° C.
45 ,, 25° C.

A positive infection experiment by means of A. lectularia.

3. IV. 1908. Seventeen bugs were allowed to feed on an infected mouse (A) showing many *Sp. obermeieri* in its blood. The bugs were allowed to feed partially for about 5 minutes and were then transferred directly to a normal mouse (B) upon which they fed to repletion.

6. IV. 1908. Eighteen bugs fed similarly on an infected mouse (C) and immediately afterwards on mouse (B).

4—14. IV. 1908. The blood of the mouse (B) was examined for spirochaetes on 11 successive days with negative results.

15. IV. 1908. Spirochaetes were found in small numbers in the blood of mouse (B).

27. IV. 1908. Mouse (B) was found dead, no spirochaetes were found on microscopic examination but their presence was proved by the inoculation of the mouse's spleen pulp and blood into another mouse, which showed spirochaetes in its blood 9. V. and died 10. V. 1908.

Conclusions (II).

The foregoing experiments, whilst not sufficiently numerous to permit of any final conclusions, appear to indicate that *Spirochaeta obermeieri* may die out more rapidly than *Sp. duttoni* in the gut of the bug. It is possible that the bugs digested their food more rapidly owing to their being more hungry than the lot used for experiments with *Sp. duttoni*. The experiments are being repeated.

It has been demonstrated by one experiment that *Acanthia lectularia* fed on an infected mouse and immediately afterwards upon a healthy mouse is capable of transmitting the spirochaete.

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THE STRUCTURE AND BIOLOGY OF *HAEMAPHYSALIS PUNCTATA*, CANESTRINI AND FANZAGO. I.

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Plates XII—XVI.

Introduction.

The importance of various species of ticks in relation to the propagation of protozoal diseases, is so generally recognised that it has appeared to us eminently desirable to make a detailed study of one of the common species. Such a study has seemed to us specially necessary for the reason that our knowledge of these parasites is very imperfect, in spite of the fact that they possess an economic interest of the first order. Some of the diseases which ticks transmit, notably those due to the haematozoal parasites belonging to the genus *Piroplasma*, are among the most devastating affections of domesticated animals in many parts of the world, the useful animals which suffer from piroplasmosis being cattle, sheep, goats, horses, and dogs. The disease known as "Heart-water," occurring in South Africa and affecting sheep, goats and cattle, is likewise tick-transmitted. A disease of the domesticated fowl, analogous to relapsing fever in man, likewise of economic importance and occurring in different parts of the world, has also been demonstrated to be transmitted from animal to animal through the agency of ticks. The fowl disease is due to a *Spirochaeta* which is conveyed by ticks; the same holds for human "tick fever" and a spirochaete infection in cattle occurring in parts of Africa. Recent investigation appears to have clearly established the fact that a tick conveys spotted or Rocky Mountain Fever to man. Moreover it has been claimed that a Nematode worm, the *Filaria perstans*, parasitic in man, undergoes its development in a tick which is capable of conveying the parasite from one human

host to another. There can be no doubt but that ticks will be found, upon further investigation, to be associated in the transmission of an increasing number of diseases in animals.

Ticks belong to the class *Arachnoidea*, in which are included the scorpions, spiders and mites, and almost all the members of the class possess eight legs when they reach the adult stage. Ticks constitute a well-defined super-family, the *Ixodoidea*, in the order *Acarina*, and in the light of recent knowledge may be divided into two families, the *Argasidae* and the *Ixodidae*.

It would be out of place to dwell here upon the particular characters possessed by the different genera, especially as these will be duly considered in a systematic work which is being prepared for publication.

The *Ixodoidea*, as far as is known, derive their nourishment entirely by sucking the blood of their hosts. These hosts are almost exclusively terrestrial vertebrates and include practically all classes of Mammalia, Aves, Reptilia (*Chelonia*, *Lacertilia* and *Ophidia*), and Amphibia (*Bufo*), Two species are recorded as attacking Insecta (*Coleoptera*)[1].

The *Ixodoidea* are distinguished from other *Acarina* by their relatively large size; by the position of the spiracles (absent in the larva), situated as they are, in the posterior half of the body, behind the last pair of legs, or as in the *Argasidae* usually between the third and fourth pair of legs; and by the unique structure of the piercing and sucking mouth parts, complicated by the fusion of the basal segments of some of the perioral appendages so as to form a definite structure, the *capitulum*, articulating with the anterior end of the body. The *body* is compressed dorso-ventrally. The *capitulum* bears two pairs of jointed appendages. On the whole, the *Ixodoidea* are found to agree in their general morphology with the remainder of the order *Acarina*, in so much as the cephalothorax is indistinguishably fused with the abdomen, in the possession of an unsegmented body and in the character of the mouth parts.

The *Ixodidae* resemble each other in all essential characters, but differ from the *Argasidae* in the fact that a hard dorsal shield or *scutum*, absent in the latter, is invariably present in all stages; also the *capitulum* is visible from the dorsal surface in the former, whereas in the latter it is entirely concealed (excepting in the larval stage) by the overhanging anterior extremity of the body: the two families differ also in many respects regarding their habits.

The life-history of the *Ixodidae* may be briefly summarised as follows:—

[1] Neumann 1901, pp. 274 and 294.

The female lays her numerous eggs in the ground. The eggs hatch out in due course and the six-legged larvae issue. The larvae attach themselves to a host and engorge blood, after which, in some cases, they fall to the ground, in others, they remain attached to the host. In all cases, they moult after a time and the eight-legged nymph appears. The nymph either attacks a fresh host or reattaches itself to the original host; it feeds and when gorged drops off, or in certain species, retains its hold. A second metamorphosis takes place and the adult tick, male or female, issues from the nymphal skin.

Copulation takes place upon the host, in some cases (*Ixodes*) it may also take place upon the ground. The males suck blood in moderate amount (a fact often denied), whilst the females become greatly distended with blood which they digest, partially while on the host and partially after dropping to the ground, after which, they lay their eggs in due course, shrivel and die. We shall consider the life-history in detail in our study of *Haemaphysalis punctata*.

Although the genus *Ixodes* is usually regarded as typical of the *Ixodidae* to which it gives its name, we have, for reasons of convenience, chosen a species of the genus *Haemaphysalis* to work upon; we have been able to raise it without difficulty through its various stages under laboratory conditions; also, the species is sufficiently common in Europe to be readily obtained. Most of our material was collected in Kent, the gorged males and females being taken, for the most part, from sheep in the neighbourhood of Romney Marsh.

Before proceeding to a further consideration of the structure of *H. punctata*, we would state that, in common with other *Ixodidae*, the main distinctions between the different stages are as follows:—

The *larva* is a minute six-legged creature, possessing no respiratory (tracheal) system; it has no trace of sexual organs. The scutum covers but a part of the dorsum. When fully gorged, it is about as large as the nymph which subsequently issues.

The *nymph* is larger than the unfed larva; it possesses a tracheal system and a pair of spiracles; also an *Anlage* of the genital system, in the shape of a minute pit at the site of the future genital orifice. The scutum approximates to that of the female. When gorged, it is almost as large as the adult which issues from the nymphal skin. We have been unable to determine any sexual differences in the larval or nymphal stages, but it may be mentioned that nymphs have frequently been mistaken by various authors for "young" females.

The *adult* is larger than the nymph and possesses a well-developed

respiratory system and sexual organs. Both sexes of the ungorged adults are of about the same size; but when fully fed, the female greatly exceeds the male in size. The scutum in the male covers almost the entire dorsum; the anterior portion only, in the female.

In species of *Ixodidae* which possess eyes, they are present, as far as we know, in all the stages, but eyes do not occur at all in the genera *Haemaphysalis*, *Ixodes* and *Aponomma*.

We shall proceed to give a somewhat detailed specific description of *Haemaphysalis punctata*, together with the iconography and distribution.

Haemaphysalis punctata, Canestrini and Fanzago, 1877.

Synonymy.

Haemaphysalis punctata, Canestrini and Fanzago, 1877.
Haemaphysalis sulcata, Canestrini and Fanzago, 1877.
Rhipicephalus expositicius, L. Koch, 1877.
Herpetobia sulcata, Canestrini, 1890.

The foregoing synonymy is given by Neumann (1897, p. 327). He includes *Haemaphysalis peregrinus*, Pickard-Cambridge (1889, p. 406), but on referring to the descriptions and figures of the latter author, we were unable to agree with Neumann, for the reason that it was impossible to recognise the tick from the inadequate description. The types, moreover, have been lost.

Haemaphysalis cinnaberina, C. L. Koch, 1844 and 1847, was degraded to *H. punctata* var. *cinnaberina* by Neumann (1905, p. 237), *vide infra*.

Canestrini (1890, p. 526) included *Ixodes chelifer*, Mégnin, 1880, and *Pseudixodes holsatus*, Haller, 1882, in this synonymy, but he was wrong in so doing, as was pointed out by Neumann (1897, pp. 338, 360).

Railliet (1895, p. 714) quotes Canestrini. Neumann (1901, p. 260) considers that *Ixodes testudinarius*, Murray (1877, p. 192), which Murray makes synonymous with *Ixodes marginatus*, Leach, to be probably *H. punctata*. He appears to base this opinion on a figure of Murray's which seems to us to be quite unrecognisable.

Description and Iconography.

Canestrini and Fanzago's original description (1877, p. 121 repr. and 1877—78, p. 189) refers only to the ♂ and ♀, and is so brief that it would be impossible to identify the species by it. The description

given by L. Koch (1877, pp. 196—198) of *Rhipicephalus expositicius* certainly agrees very closely with that of *H. punctata*, but he gives no figure. Canestrini (1890, pp. 523—526) subsequently gave a more detailed and fairly accurate description, more especially of the ♀; he gives the first figures published (Pl. 41, Figs 6 and 6*a*), showing a ventral view of the ♂ and a ventral view of the ♂ capitulum. Although inaccurate, Canestrini's figures show the principal ♂ characters. All the points in his description, with the exception of a few measurements, are included in Neumann's description (1897, pp. 327—330). Berlese (1891, Fasc. 55, Pl. 1) gives a coloured figure of *H. sulcata*, together with outlines of the dorsal and ventral aspects of the capitulum and of the spiracle. To us there appears to be no doubt that he was dealing with a gorged nymph of *H. punctata*, although he omits the cornua on the basis capituli and places the chelicerae ventrally instead of dorsally in his figure of the capitulum. In a later publication (1891, Fasc. 58, Pl. 10) he gives an inaccurate coloured figure of *H. punctata* ♂, and equally inaccurate contour figures of the ♂ (ventral aspect), ♂ capitulum and palp, ♀ (dorsal and ventral), ♂ and ♀ spiracles. Finally, Neumann (1897, Figs. 1 and 2, p. 328) figures the hypostome accurately and gives dorsal views of the ♂ and ♀ chelicerae of the left side. His figure of the ♂ chelicera is correct (compare with our Pl. XIV, Fig. 4 and XV, Figs. 5, 6) but that of the ♀ is inaccurate, more particularly with regard to the so-called third apophysis (dorsal process). Wheler (1906, Figs. 30—32) has published photographs of the ♂ and ♀.

Specific Description.

Female (Plate XIII).

Unfed. Length (anterior margin of scutum to posterior margin) 2·73—3·23 mm. Breadth (maximum), 1·78—2·08 mm.

Body[1] flattened, contour ellipsoidal; reddish-brown dorsally, paler ventrally; glabrous (few excessively minute scattered hairs); *marginal grooves* deep, sharply defined, extending from external angles of scutum, anteriorly, to 4th festoon on either side, posteriorly: *postero-median groove* shallow, extends from median festoon almost to posterior margin of scutum; *accessory grooves* as long as *postero-median groove*, wide and shallow: *foveae* opposite legs IV, darker in colour than surrounding

[1] Refer to text figures 1, 2, p. 165.

parts: *festoons* rectangular, twice as broad as long, nos. 1, 2, and 3 only, on each side, divided by the marginal groove from rest of body. *Scutum*, length 1·08—1·37 mm., breadth 1·05—1·31 mm.; broadly elliptical with flattened postero-lateral margins; dark reddish-brown with darker irregular markings; deeply emarginate anteriorly for reception of basis capituli; surface polished, traversed by minute irregular radiating furrows; punctations irregularly scattered, few in number: *cervical grooves* deep anteriorly, shallow and wider posteriorly, almost parallel throughout their length. *Venter*:—*spiracles* nearly circular, external margin flattened, macula large and slightly excentric: *genital grooves* divergent, do not quite reach festoons posteriorly: *genital orifice* opposite interspace between Coxae II and III: *anus* nearly circular; *valves*[1] punctate and bearing 5 minute hairs on each: *anal groove* well-marked, bifurcated at its anterior extremity in the form of a Y, the forks of which encircle the anus on either side and terminate in the genital grooves.

Capitulum (see text figs. 3—9, p. 169 et seq.): length, 770—880μ (from line joining tips of palps to line joining extreme postero-lateral angles of basis capituli): *basis capituli* rectangular, cornua obsolete; *porose areas* large, well separated, rather wider than long; ridge on ventral surface semicircular in contour: *palps* valvate, sub-conical, truncated; article 1 small, almost entirely concealed within the basis capituli, article 2 contracted proximally, much widened out distally with a prominent ridge running round its ventral, lateral and dorsal surfaces and forming the external angle of the palp; infra-internal margin furnished with a continuous fringe of feather-like hairs, usually thirteen in number but subject to some variation; three similar hairs on corresponding margin of third article, the ventral surface of which is produced as a large pointed backwardly directed protuberance; article 4 small and stumpy, with a tuft of stiff hairs on its free extremity, Neumann (1897, p. 328) gives the number of these hairs on the fourth segment of the palp as six; we have found eight to ten such hairs, but the number does not appear to be by any means constant): *chelicerae*: *internal article* with large base and prolonged as a single cutting-lancet with an outwardly turned point; *dorsal process* crescentic with the points folded towards each other; *external article* a flattened plate with four (sometimes five) outwardly directed cusps, the first of which is the smallest, the others gradually increasing in size to the largest

[1] Neumann (1897, p. 328) says that the anal valves are punctate, but without hairs: we have invariably found five minute hairs on each valve in addition to punctations.

proximal cusp: *hypostome* spatulate, five files of sharply pointed teeth on each half (we have found a slight degree of variation in this respect, the specimen selected for the figure, Pl. XIV, Fig. 2, possessing an irregular sixth file on the right half of the hypostome; we have also seen a female of *H. punctata* possessing six complete rows of teeth on either half of the hypostome); the anterior, median and posterior teeth are smaller than the rest; *corona* well-developed, studded with minute denticles (the corona is frequently curled over on to the dorsal surface of the hypostome and may escape observation if the curling is extreme).

Legs of moderate length, dark-brown, strong; *coxae* slightly longer than wide, each bearing a short wide spur at the posterior margin, near the internal angle; *tarsi* relatively short, spurred, the single spur on each being terminal: *pulvilli* of medium size; two-thirds length of claws. (Canestrini (1890) gives the length of the legs as follows: Pair I = 2·95 mm., II = 2·50 mm., III = 2·5 mm., IV = 2 mm.)

Gorged (Pl. XIII). *Body* oval, more or less distended, may attain a size of 12 × 7·5 mm. (Canestrini, 1890); colour variable—light steel-grey, slate-grey, brownish or brownish-black, yellowish streaks and irregular markings present according to the condition of distension of the Malpighian tubes; dorsal and ventral surfaces punctate: *marginal grooves* and *festoons* obliterated; *postero-median* and *accessory grooves* well defined; anterior to these, four short almost parallel grooves, the outermost of which are often interrupted at the middle of their length (Pl. XIII). *Venter:—post-anal groove* not so sharply defined as in unfed ♀ and its bifurcated anterior extremity is obliterated: two dimple-like depressions, one on either side of post-anal groove: *genital orifice* slightly anterior to level of *coxae II*, but position varies according to the state of engorgement, being pushed more and more forward until oviposition commences, when it lies nearly at the base of the capitulum. Otherwise as in ungorged ♀.

Male (Plate XIII).

Unfed. Length (measured as in female) 2·6—3·27 mm., breadth (maximum) 1·67—2·04 mm.

Body ellipsoidal in contour, wider behind than in front, convex dorsally, concave ventrally: *scutum* covers entire dorsal surface; dark reddish-brown with darker, almost black irregular markings; glabrous and polished; punctations fine, numerous, regularly distributed; *scapular angles* narrow; *cervical grooves* deep anteriorly, shallow and divergent posteriorly; *marginal grooves* narrow, sharply defined,

terminating anteriorly opposite leg II, posteriorly at 4th festoon; *accessory grooves* shallow, deeper at posterior extremity; *foveae* in small depressions opposite interspace between legs III and IV. *Venter:*—*spiracles* oval, wider in front than behind, external margin almost straight, postero-marginal angle pronounced; *macula* small, situated towards antero-internal angle: *genital grooves* almost parallel anteriorly, divergent behind coxae IV, extend to posterior marginal festoons; *post-anal groove* straight, bifurcated at its anterior end as in female: *festoons* sharply divided from body by a deep groove: *genital orifice* opposite coxae II: *anus* as in female.

Capitulum, length 500—670μ (measured as in female): *basis capituli* rectangular, relatively longer than that of female; dorsal ridge with salient rounded angles (cornua) at its extremities; few coarse scattered pores on site of porose areas of female: *palps* relatively shorter than those of the female, more conical and less truncated; retrograde spine on ventral surface of article 3 more pronounced: *chelicerae—internal article* as in female; *dorsal process* with single large outwardly directed cusp from which a thin strap-like process runs down the dorso-external margin of the internal article and becomes indistinguishably fused with it; *external article* small, with three cusps, the most proximal of which is large and strong: *hypostome* less spatulate than that of female, otherwise similar.

Legs I—III with well-marked blunt *coxal spurs*; leg IV with elongated sabre-shaped coxal spur directed backwards and curving inwards, equal in length at least to that of the coxa: a short blunt backwardly directed spur on the dorsal side of the trochanter of leg I; *tarsi* as in female; Neumann (1897) observes that the tarsi IV of the ♂ *Haemaphysalis punctata* are bicalcarate, a point which we have failed to confirm.

Gorged (Pl. XIII). Alteration so slight as not to necessitate description.

Nymph (Plate XIII).

Unfed. Length: tips of palps to posterior margin 1·39—1·55 mm. (measured as in adult), breadth 1·12—1·31 mm.

Body—oval, flattened, colour varying from pale yellow to brownish-red: *scutum* sometimes broader than long (length 0·37—0·55 mm., breadth, 0·47—0·57 mm.); more rounded than that of ♀; few scattered punctations; divided into minute polygonal areas by a fine reticulum of superficial fissures; otherwise like that of ♀: *grooves* on dorsum similar

to those of ♀ : *foveae* extremely small. *Venter* resembles that of female, the *genital orifice* being represented by a small imperforate pit: *spiracles* almost circular ; *macula* excentric, situated towards the inner margin of the spiracle.

Capitulum—length (from tips of palps to postero-dorsal ridge) 0·23—0·29 mm.: *basis capituli* widest at base of palps; dorsal surface extended laterally on either side as a triangular process with rounded point ; *porose areas* absent: *palps* longer than hypostome, of elongated triangular form; prominent external angle on article 2, which bears on its infra-internal margin a fringe of about 5 feather-like hairs; article 3 carries a single feather-like hair in a corresponding position : *chelicerae —digit* as in female: *hypostome* slightly spatulate; two files of teeth on each half.

Legs paler in colour than in adult; no tarsal spurs; otherwise as in adult.

Gorged. The changes undergone by the nymph during engorgement are similar to those of the ♀, viz. obliteration of the grooves on the surface of the body, alterations in colour, etc. When replete the nymph measures 3·3 × 2 mm.

Larva (Plate XII).

Unfed. Length (tips of palps to posterior margin) 0·58—0·70 mm., breadth (maximum) 0·43—0·48 mm.

Body oval, wider posteriorly, less flattened than other stages; colour dull ochre-yellow ; few pale-coloured hairs, regularly arranged (see Figs. 1 and 2) ; *scutum* broader than long (length, 0·25 mm., breadth, 0·32 mm.); reddish-brown in colour, contour follows that of body in anterior half, postero-lateral margins almost straight, with large rounded postero-median angle; surface reticulated as in nymph; *cervical grooves* deep, straight and not extending to posterior margin : *foveae* absent ; *postero-median* and *accessory* grooves faintly marked. *Venter:—post-anal groove* slight; *genital grooves* absent: *spiracles* absent.

Capitulum, length (tips of palps to dorsal ridge) 0·15 mm.: *basis capituli* as in nymph but lateral angles less pronounced: *palps* tri-angular, external angle not prominent; all articles fused together with the exception of the 4th, which is relatively larger and more terminal in position than in the other stages; a single feather-like hair is carried on the infra-internal margin : digits of *chelicerae* as in nymph and female: *hypostome* with four files of teeth, two on each half.

Legs, pale ochre-yellow; no spurs on coxae or tarsi; *pulvilli* relatively larger than in other stages.

Gorged. Beyond the distension of the body, the changes during and after engorgement in the larva do not call for any special reference. A noteworthy feature is the fact that larvae of *H. punctata*, taken from inside the ears of rabbits, when fully gorged are a clear straw-yellow in colour, from which it would appear that lymph and not blood had been ingested.

Haemaphysalis punctata var. **cinnaberina** (C. L. Koch), Neumann 1905.

This variety, based on a single young ♀, is distinguished from the typical *H. punctata* by the presence of a white chitinous circle round the genital orifice and also round the anus. The type, a dried specimen, from Pará, Brazil, is in the R. Lucas Collection (Berlin Mus.) where it was examined by Neumann (1897, p. 331), who first described it as *H. cinnaberina*, C. L. Koch, but subsequently degraded it to a variety. This tick was inadequately described and figured by C. L. Koch (1844, p. 237; 1847, p. 123 and Pl. XXVI, fig. 97).

Geographical Distribution of *Haemaphysalis punctata*. Hosts attacked.

Canestrini (1890, p. 525) and Berlese say that this species is fairly common on sheep in Italy; also on goats and fallow deer. L. Koch found a ♂ in a wood near Nürnberg, and named it *Rhipicephalus expositicius*. Neumann has males collected by Colin on a bull (Railliet Coll.): one ♂ and 4 ♀'s from Digne, Beaune, Corsica (Simon Coll.): 2 ♀'s and 1 ♂ from Villefranche found on a horse (Aveyron): 5 ♀'s and 6 ♂'s from sheep and ox at Ste. Jean de Luz: 1 ♀ and many larvae from sheep in England (B. A. I. Coll., Washington, D.C.): 1 ♀ on *Numenius arquata* and 1 nymph on *Otus otus* at Utrecht (Oudemans Coll.): 2 ♀'s at Jassy, Roumania (Leon Coll.): 1 at Fiume, Croatia (Simon Coll.): 1 found under stones and 1 ♀ on a lizard, *Acanthodactylus vulgaris* at Oran (Domergue): 1 ♀ on a bull at Blida, another on a goat at Medea, 1 from Marnia, 1 from a sheep in Egypt (Smithsonian Inst. Coll. Washington): 1 ♂ and 1 ♀ from Orotava, Teneriffe, and 1 ♀ from Funchal, Madeira (Kraepelin Coll. Hamburg Mus.): 5 ♀'s from Canary Islands (Paris Mus.): 1 ♀ from a horse in Aomori, Japan. According

to Neumann, *H. punctata* is sufficiently cosmopolitan but does not appear with frequency; the specimens collected are never very numerous. Canestrini and Berlese found nymph and larvae in Italy, on *Lacerta viridis* and in Dalmatia on *Lacerta muralis* var. *pelagosae.* Neumann's specimens came from a lizard in the Island of Cyprus, and from horse, hare, red partridge and grey partridge (Aveyron).

Since Neumann's description appeared, *H. punctata* has again been recorded in England by Pocock (1900, p. 326), who states that the specimens were collected by Mr F. Pickard-Cambridge at Dungeness, on a hedgehog and amongst shingle on the beach. Neumann (1901, p. 260) records 11 ♀'s, in 5 lots, of which one lot is at the Hamburg Museum and four at the Berlin Museum, which were derived from Athens, Crete, the Cyclades, Teneriffe and Japan: 9 nymphs found on *Lacerta ocellata* var. *tingitana*, were collected by Domergue at Djebel Ksel in Algeria: 3 nymphs were found on *Vipera aspis* by C. Parona at Genoa; 1 ♀ found on the hedgehog by Oudemans in Holland.

In the course of the last few years, we have received large numbers of specimens from Kent, especially the districts surrounding Lydd and Canterbury: the great majority of these were taken from sheep, but specimens taken from goats and ferrets have been received.

Technique and Methods.

In the study of the external anatomy of *Haemaphysalis punctata*, the authors have examined living specimens; killed, cleared and mounted entire in various media; dissections and sections. The coloured figures are faithful reproductions of living specimens, the original drawings of which were made by means of the Abbe camera lucida and amplified by numerous sketches of mounted specimens.

The Zeiss binocular dissecting microscope has proved itself of the greatest value in the making of fine dissections and in the execution of sketches of the living tick.

Specimens intended for the study of the structure of the chitinous exoskeleton were usually treated with a cold 10 % solution of potash for some hours, or until the soft parts were entirely dissolved, washed in distilled water containing a trace of acetic acid, stained with picric acid, orange G or osmic acid (1 % aqueous solution), dehydrated by transference through graded alcohols, cleared in xylol or oil of cloves and mounted in Canada balsam. It was found that the chitin was efficiently stained by immersion for one minute in xylol saturated with

picric acid, immediately before the final clearing. Absolute phenol has been of great use in clearing entire specimens, the procedure being to melt a small quantity in a bottle or a watchglass in the embedding oven; drop the specimen in, leaving it until sufficiently cleared; transfer to xylol and mount in Canada balsam. The advantages of this method are the saving of time, the clean results, and the fact that the specimen is completely cleared before the soft parts are disintegrated—a useful feature in the study of the relationship between the exoskeleton and the internal structures.

Glycerine, glycerine jelly and especially monobromide of naphthalene have been used with advantage as mounting media in the study of the structure of the almost colourless, highly refractive chitinous parts, such as the digits of the chelicerae and the pulvilli. Minute dissections were made, as a rule, in oil of cloves, a method which facilitates manipulation with mounted needles. In the preparation of material for section cutting, all the better known fixing reagents have been tried, but the majority rendered the chitin so brittle that success was impossible. The reagent which has given satisfactory results is picro-sulphuric acid (Kleinenberg's formula). This is used in the undiluted condition and is heated to about 90° C. before immersing the material. The time required for complete penetration varies, but gorged females of *H. punctata* have been found to be thoroughly permeated with the reagent after an immersion of 1—2 minutes.

The authors intend to consider the subject in greater detail in the section dealing with the internal anatomy and histology.

For many purposes, photomicrography has lent assistance, but with few exceptions drawings alone have been used for illustration.

In the work on the structure of the external parts, considerable difficulty was experienced in obtaining proper orientation and fixation of the more minute parts for the purpose of drawing: this led the authors to try various plastic materials in which the part to be investigated could be lightly embedded and then orientated under the microscope, by means of mounted needles. The preparation known as Plasticine has proved an admirable medium for this purpose, and its use can be recommended in all cases where it is desirable to examine opaque objects which, on account of their form, are difficult or impossible to fix in proper position without resort to some such means.

TERMINOLOGY.

One of the first features which strikes a reader, in perusing the published literature, is the hopeless confusion wrought by the loose use of terms, taken for the most part from the nomenclature used in the description of other classes of the animal kingdom, more particularly the Insecta.

As an example one might mention the *hypostome*, to which names innumerable have been applied, many of them being unsupported by morphological relationship; thus creating erroneous impressions of the homologies of this structure. Thus the *hypostome* has been called:—

(1) *Lingua.*	(5) *Maxilla.*
(2) *Maxillo-labial dart.*	(6) *Labium.*
(3) *Glossoide.*	(7) *Radula.*
(4) *Ligula.*	(8) *Languette.*

With a view of obviating some of this confusion, we have been reluctantly compelled to add more terms to the already excessive number: in other cases a selection has been made from the existing nomenclature of those which have appeared to be the most suitable.

A list of the terms used, together with synonyms, will be appended at the end of this work.

General Body-form.

Haemaphysalis punctata presents no marked structural differences from the rest of the *Ixodidae*. The *body* is roughly elliptical in contour, flattened dorso-ventrally, slightly concave on the ventral surface and convex on the dorsal surface in unfed specimens. It shows no differentiation into *cephalothorax* and *abdomen*. Deeply implanted into a special opening at the anterior end of the body is the *capitulum*: this structure, frequently referred to as the "head," is not such in a morphological sense, the true head of the tick comprising parts lying posterior to this and indistinguishably fused with the rest of the body. The *capitulum* is a very specialised structure, freely articulated with the body and bearing the oral opening together with its accompanying appendages. The *mouth* is not visible on external examination, being concealed between the median *hypostome*, ventrally, the *palps* laterally, and the paired *chelicerae*, dorsally. The *palps* are situated on either side of the *hypostome* and *chelicerae* and when adducted, ensheath and protect these medianally-placed appendages.

Key Figures to nomenclature of parts.

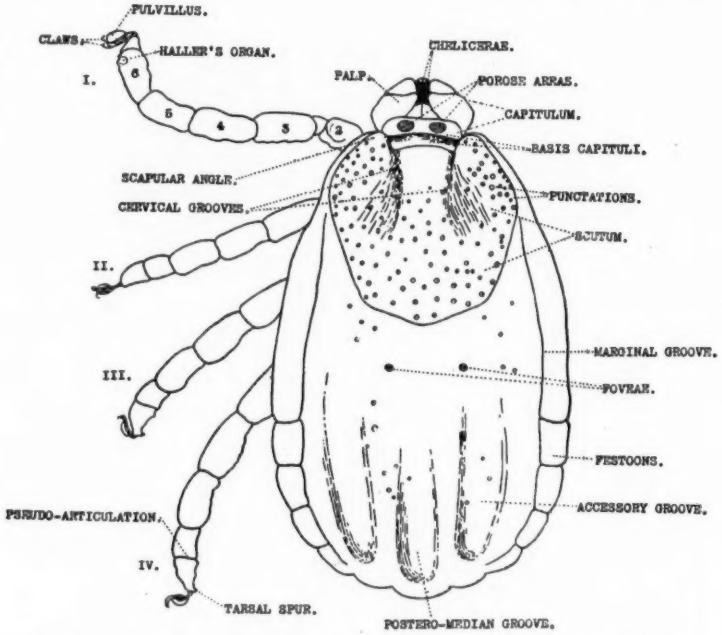


Fig. 1. *Haemaphysalis punctata*. ♀. Dorsal aspect showing the principal external features. The capitulum, on account of its downward inclination, is foreshortened. L.E.R.

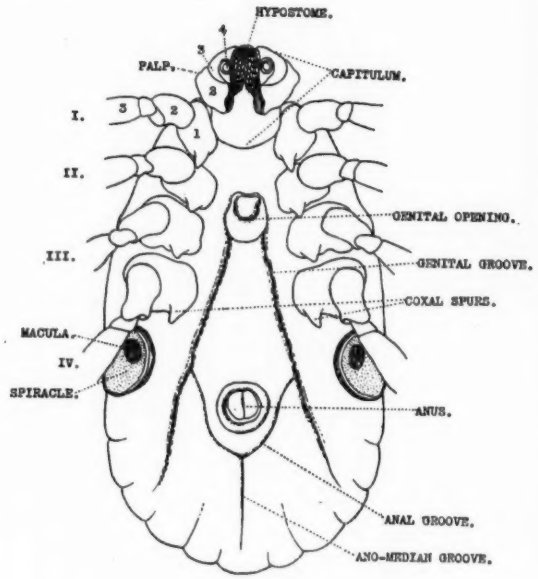


Fig. 2. *Haemaphysalis punctata*. ♀. Ventral aspect. L.E.R.

The roman numerals indicate the legs, the arabic figures the segments, of the appendages.

It may be said that the body shows no marked trace of segmentation in either the nymphal or adult stages, but in the larva at any rate, the arrangement of the hairs and pores indicates a certain amount of metamerism; possibly the crowding of these tegumental structures in the later stages of the life-history may obscure any such appearance. The regular postero-marginal indentations of the body-contour in all the stages also seem to point to a feeble persistence of segmentation of the abdomen. We have reason to believe, after an examination of the embryo of this species, that at an early period of the development, some five or six segments appear in the body, posterior to the last pair of limb-buds. The exact significance of these phenomena we hope to determine later, when we have an opportunity of devoting more time to the embryology of the *Ixodoidea*[1].

The dorsal surface of the body is covered almost entirely in the male and the anterior half only in the female, nymph and larva, with a plate of chitin which is denser, harder and more deeply pigmented than the general body chitin; this structure which is present in all ticks with the exception of the *Argasidae* is termed the *scutum*.

In many genera of ticks, the scutum bears the single pair of eyes, one on either lateral margin; in *Haemaphysalis*, however, as in the genera *Ixodes* and *Aponomma*, no trace of these organs exists.

On the ventral surface of the body in its anterior half are borne the legs, four pairs in the adult and nymph, and three pairs in the larva. Each leg is comprised of about six articles, all of which are freely movable with the exception of the most proximal article or *coxa*. Each of the legs carries at its extremity a foot provided with two long curved claws (*ungues*) and a pad (*pulvillus*).

On the ventral surface of the body in the adult, two large openings are seen in the middle line. The first lies midway between the coxae of the first and second pairs of legs and has the appearance of a transverse slit: this is the *genital orifice*. In the nymph, a minute pit is visible in a corresponding situation which may be considered as an *Anlage* of the genital orifice of the adult. The larva exhibits no trace of such a structure. Some distance posterior to the genital opening, at about the commencement of the posterior third of the body, is the *anus*, a longitudinal slit bounded laterally by a pair of crescentic plates, the *anal valves*, the whole being surrounded by a ring or annulus of thickened chitin.

[1] Wagner J. 1892, p. 319 "*Hinter den Beinen liegen 5—6 Mesodermgruppen.*"

The surface of the body exhibits a number of constantly occurring and well-defined grooves: these are the external indications of the lines of insertion of bands of muscle-fibres which run in a dorso-ventral direction through the body and are attached at either end to the chitinous cuticle. On the ventro-lateral margins of the body, posterior to the coxae of the fourth pair of legs, are situated the paired *spiracles*, a pair of more or less circular, slightly raised plaques of paler colour than the surrounding integument: in the larva, the spiracles and tracheal system are not developed. The posterior contour of the body is deeply indented at regular intervals by short grooves; these run round the margin from the dorsal to the ventral surface and define certain small rectangular marginal areas which are termed *festoons* in descriptive nomenclature. It is worthy of notice that the festoons are frequently more or less obliterated in the female and immature stages, by the distension of the body after engorgement, but although the alteration is not so marked in the male, the appearance of the festoons differs considerably in the unfed and gorged individuals (Pl. XIII). The *cuticle* of the tick is perforated by numerous pores of various forms which serve as a means of communication between certain modified hypodermal cells and the exterior. Numerous hairs are distributed over the different parts of the body; these differ among themselves according to their various functions. Certain areas of the body are glabrous.

The sensory organs are comprised of (1) certain defined areas into which numerous fine pores open, and beneath which the hypodermal layer is specially modified: such organs are found in the female on the dorsal surface of the basis capituli (*porose areas*), and in both sexes on the dorsum, where a pair of minute perforated areas are found, to which the name of *foveae* has been applied; these foveae the authors have found to exist in the nymph but not in the larva: (2) *Haller's organ*, a complex cavity in the cuticle of the tarsal portion of the first pair of legs, which communicates with the exterior by a minute slit-like pore and bears on its floor a number of sensory hairs, but does not contain otoliths[1]: (3) *tactile hairs* of different forms, arranged either in a scattered manner or collected into groups.

The tactile sense appears to be the most highly developed, after that the olfactory sense, and in spite of the absence of eyes, as found in some genera of *Ixodoidea*, *H. punctata* is very sensitive to light and in-

[1] We intend to publish an account of the general structure of "Haller's Organ" in different species, in the near future.

variably attempts to remove itself as far as possible from a source of light if exposed.

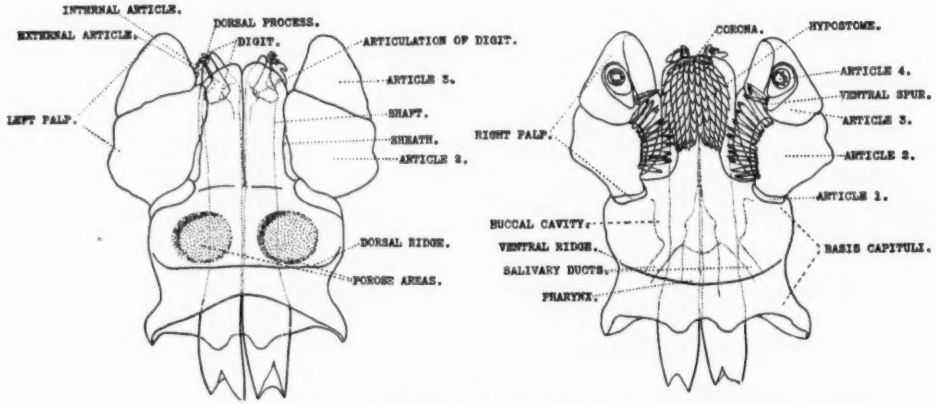
It must be borne in mind, especially in the reading of specific descriptions of *Ixodoidea*, that the general appearance undergoes very considerable transformations during and after engorgement; this is most noticeable in the larva, nymph and female, but the male may be considerably altered (see Pl. XIII). In the matter of colour, the gorged female exhibits any colour from pale greyish-yellow to almost pitchy-black. Prolonged immersion in preservatives, especially in alcohol, may completely modify the original colour, gorged females of *H. punctata* usually acquiring a more or less uniform dark reddish-brown tint.

I. The Capitulum of the Female.

(Plate XIV, Figs. 1 and 2, Text Figs. 3—5.)

The capitulum is the head-like structure, situated at the anterior extremity of the body, upon which is borne the oral opening with its accompanying appendages. The large basal portion, hereafter spoken of as the *basis capituli* (b.c.), is constricted in its posterior half and this neck-like portion is telescoped into the anterior opening of the body cavity. The anterior portion of the basis capituli is roughly rectangular in shape, with flattened surfaces dorsally and laterally, and somewhat convex ventrally. A pronounced salient ridge, the *dorsal ridge* (d.r.), runs transversely across the dorsal surface, which, when the capitulum is raised in a line with the body-axis, comes in contact with the anterior margin of the scutum, the posterior constricted portion being concealed beneath the latter. Ventrally, the anterior portion of the basis capituli is differentiated by another ridge, the *ventral ridge* (v.r.), running in a transverse direction and curved, with its convexity directed posteriorly but not so sharply raised as the dorsal ridge. The sides of the basis capituli are flattened and the dorsal and ventral ridges are not continuous.

At the antero-lateral angles of the basis capituli, deeply implanted into a pair of large rectangular fenestrae, are the *palps* (p.). The ventral surface of the basis capituli is produced anteriorly in the median line as a spoon-shaped prolongation beneath the mouth; this structure is termed the *hypostome* (h.). On its dorsal side, the basis capituli is prolonged between the palps as a pair of parallel tubes which ensheath the *chelicerae*. The dorsal surface shows a pair of large shallow saucer-like



Figs. 3 and 4. *Haemaphysalis punctata.* ♀. The capitulum seen from the dorsal and ventral aspects respectively. L. E. R.

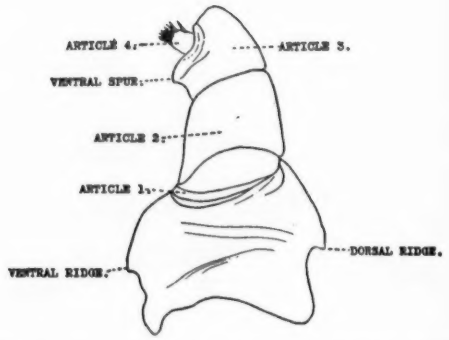


Fig. 5. *Haemaphysalis punctata.* ♀. Lateral aspect of the capitulum. L. E. R.

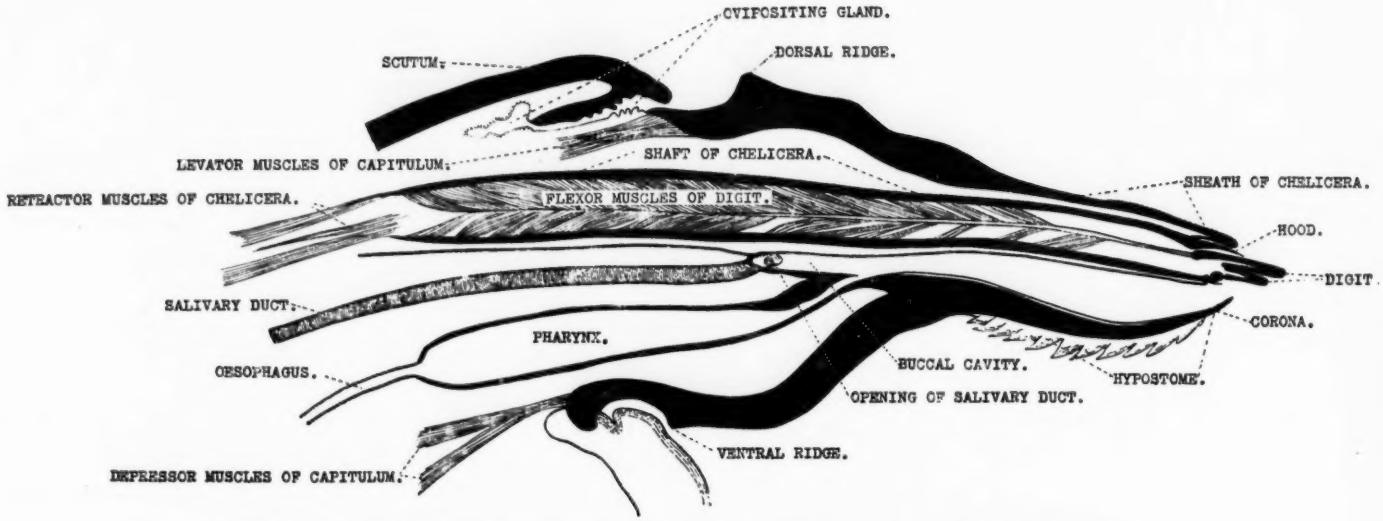


Fig. 6. *Haemaphysalis punctata*. ♀. Median longitudinal section through the capitulum showing the relations between the internal and external parts. Slightly schematized. G.H.F.N. and L.E.R.

depressions into which numerous minute pores open, hence termed the *porose areas* (p.a.); these structures will receive more detailed consideration later, under the heading of organs of the senses. With the exception of two small patches on the ventral surface, beset with minute stumpy hairs, the basis capituli is glabrous.

The chitinous integument of the basis capituli is thick and strong, and is thickened in internal ridges which give the whole structure stiffness and serve for the attachment of the muscles which bring about the movements of the capitular appendages and the *pharynx*.

The *hypostome* (h.), already referred to, is spatulate in outline and consists of a small proximal portion, whose ventral surface is smooth and convex, and a larger distal portion provided on its ventral surface with ten longitudinal files of pointed denticles which overlap one another from before backwards, in the manner of roof-tiles. It is divided along the median line by a fissure, separating the denticles into two series of five files, each file consisting of about twelve denticles. Although the number and arrangement of these denticles are fairly constant, a certain amount of irregularity frequently prevails, especially at the distal and proximal extremities. The denticles differ in size, the anterior and posterior being the smallest and the lateral being the largest. At the free extremity of the hypostome, is a thin plate of chitin continuous with the body of the hypostome, variable in size and studded with minute irregularly arranged denticles; this structure, the *corona* (c.), appears to be relatively larger in the immature stages. The upper surface of the hypostome is concave in a longitudinal direction and convex transversely; running down the median line is a small gutter (see transverse sections Plate XVI, Figs. 1—5), which, when opposed to the sheaths of the chelicerae above, forms a tube leading back to the buccal cavity. A longitudinal ridge runs along the lateral part of the ventral surface of each cheliceral sheath and a pair of slight ridges on the dorsal surface of the hypostome fit within the former, thus effecting a complete lateral closure of the space between the hypostome and chelicerae during the action of sucking (Plate XVI, Figs. 2, 3).

The *chelicerae* (Plate XV, Fig. 4 and Text Figs. 7—9) consist of a pair of more or less cylindrical appendages situated above the mouth. Each consists of a stout external *sheath* (sh.) already described as an anterior prolongation of the basis capituli, within which the chelicera proper lies, and is capable of complete retraction and of a considerable amount of protrusion: the two external sheaths lie in contact with one another throughout their length. The chelicera

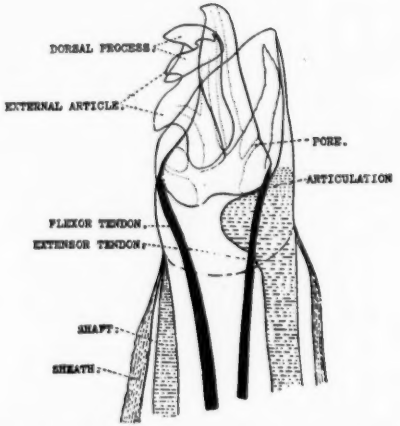


Fig. 7. *Haemaphysalis punctata.* ♀. The distal extremity of the right chelicera, showing the articulation of the digit, etc. L.E.R.

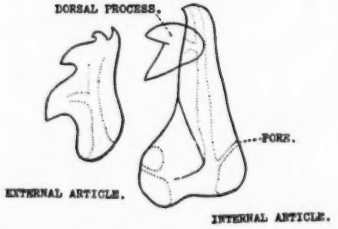


Fig. 8. *Haemaphysalis punctata.* ♀. Right chelicera, the articles isolated. L.E.R.

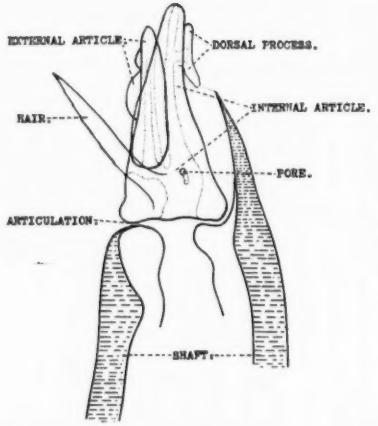


Fig. 9. *Haemaphysalis punctata.* ♀. Right chelicera, median or internal aspect. L.E.R.

proper consists of a long cylindrical tube, the *shaft* (s.ch.), upon the distal and free extremity of which is borne the chelate *digit* (d.ch.) which constitutes the cutting apparatus by means of which the animal penetrates the skin of its host. The sheath is covered on its dorsal and lateral surfaces, excepting its proximal third, with numerous minute reflexed denticles, regularly arranged in oblique rows and in close contact with one another, giving it an appearance which has suggested the term "shagreened sheath": it is firmly fused with the basis capituli at its proximal end. At its free extremity, the sheath is invaginated and becomes membranous and flexible, and this thin membranous portion is attached to the shaft some little distance posterior to the articulation of the terminal digit, by which arrangement the shaft draws in the extremity of the sheath over the delicate digital structures when fully retracted. The shaft of the chelicera is very long, so long in fact as to project backwards beyond the basis capituli into the body-cavity: it is divided into two portions, differing in diameter. The proximal portion, comprising about one-third of the entire length, has thinner walls than the distal portion and is dilated: the edges of the proximal extremity are irregularly incised and it is to this extremity that the retractor muscles of the chelicera are attached (Text Fig. 6): this dilated portion is occupied by muscles attached to its internal surface and from which proceed the tendons which are inserted into the base of the digit. The distal two-thirds of the shaft is roughly cylindrical with a slight ridge along its supra-internal margin, the ridge being longitudinally traversed by a minute canal (Plate XVI, Figs. 2—9): the chitinous walls are thick and show a delicate transverse striation: the distal extremity is thickened internally and presents a surface for the articulation of the base of the digit. At the junction of the proximal and distal portions of the shaft is a somewhat rectangular area of thinned and membranous chitin, in length about equal to the diameter of the shaft and extending round the dorso-lateral surface of the latter for about half its circumference. The *digit* consists of two movable articles, a larger *internal article* (i.a.), with which is articulated a smaller *external article* (e.a.). The internal article is expanded at its base and articulates directly with the shaft; it is prolonged forwards on the internal side in the form of a long cutting lancet with a slightly hooked extremity, the point of which is directed laterally; it is hollow throughout its length, the cavity in the expanded base being of considerable size. Near the distal extremity of this article, attached to its dorsal surface, is a crescentic chitinous structure with laterally directed points which is

often referred to as the *third apophysis*: it is firmly fused to the internal article, a prolongation of the cavity of which extends into it, and we consider it to be simply an outgrowth of the latter, comparable to similar processes often seen on the homologous articles of the chelicerae in the *Gamasidae* and *Oribatidae*, and for this reason we prefer to term it the *dorsal process* (d.p.). The external article articulates with the expanded base of the internal article; it consists of a flattened plate of chitin, the external margin of which is deeply incised, so as to form a series of four or five pointed cusps, of which the terminal one is the smallest, and the proximal the largest: it is hollow and its internal cavity is continuous with that of the internal article; indistinct channels appear to run out from the central cavity for a short distance along the lateral cusps. In many cases, the cavity of the internal article appears to terminate in a minute pore at the distal extremity; whether this is actually the case we are not in a position to express a definite opinion: it is, however, certain that the interior of the expanded base of the internal article communicates with the exterior by at least two fine channels, one on the internal side and one on the external (pr.), the possible function of which it is difficult to imagine.

The digit of the chelicera is surrounded on its internal side by a delicate hood of thin and very transparent chitin, a prolongation of the shaft of which it is a part; it is tapered away on the external side of the digit in such a manner as to fully expose the cutting parts of the appendage. The internal article bears on its basal portion a single serrate hair, which protrudes between the hood and the lancet-shaped portion of the former (see Text Figs. 7 and 9 and Pl. XV, Figs. 4—6).

The base of the digit, formed entirely from the expanded portion of the internal article, articulates with the shaft by a rocker-like hinge-joint, the shape of the articular surfaces being such as to limit the movement of the digit to a horizontal plane. The movements of the digit are produced by the tendons, one internal and one external, which originate in the mass of muscle occupying the cavity of the shaft (see Text Fig. 6, p. 170): the internal tendon is inserted into the internal article at its proximo-internal angle, while the external tendon, the stouter of the two, runs partially round the lateral surface of the article and is inserted towards the upper surface of the expanded base.

The palps. These appendages are inserted into the basis capituli at its antero-lateral angles. The general outline of each is that of a truncated cone; the basal portion is much constricted laterally, but not

in the dorso-ventral direction. Each palp is quadriarticulate, but the articles differ greatly in size and form. The proximal or *first article* is very small and is almost entirely hidden within the basis capituli; it is narrow from side to side, elongated in the dorso-ventral direction, and obliquely inclined to a plane at right angles to the long axis of the capitulum in such a manner as to bring its ventral portion posterior to the dorsal portion: externally it is only visible ventrally, being completely hidden on the dorsal side by a scroll-like fold of chitin derived from the basis capituli. The second and third articles constitute the greater portion of the palp. The *second article* is irregular in shape, very convex on its ventral, external and dorsal surfaces and is provided with an oblique ridge (r.) which runs forwards and outwards from the proximo-internal angles and which defines another surface directed backwards. The *third article*, smaller than the second, tapers distally to a blunt rounded extremity which forms the extremity of the palp; on its ventral surface, a little toward the internal margin, it bears a large pear-shaped fossa bounded by a raised margin, the latter being produced postero-internally into a large blunt salient process; in this fossa the comparatively minute *fourth article* is implanted. This article is very short and stumpy and is furnished with a crown of eight to ten short stiff hairs on its distal extremity. The interno-lateral aspect of the second and third articles of the palps is deeply concave, so that when adducted, the two palps ensheath the chelicerae and hypostome. The infra-internal margin of the palp is furnished with a single row of peculiar flattened feather-like hairs (Pl. XV, Fig. 1); of these hairs, thirteen to fifteen are carried on the second article and about three on the third article: they are directed towards the mid-ventral line of the capitulum, the tips of those of each side almost meeting when the palps are adducted. The surface of the palps is furnished with a few simple hairs and pores.

The *buccal cavity*. The buccal cavity is contained within the anterior part of the basis capituli; it lies between the bases of the palps, behind the hypostome, and is visible through the walls of the basis capituli in cleared specimens as a chitinous mass with a symmetrical outline of somewhat complex shape (the contour is indicated in Pl. XIV, Figs. 1, 2)[1]. The walls of the basis capituli, bounding the anterior

[1] On account of the prevailing system of making drawings from specimens cleared and mounted in Canada Balsam or other highly refractive medium, without reference to the untreated specimen, the outline of the buccal cavity and other internal structures have frequently been figured by different authors in illustrations to specific descriptions of the *Ixodidae*. As such figures are usually supposed to represent external structures, the inclu-

margin of the buccal cavity immediately behind the hypostome, are of great thickness, giving increased strength to the anterior part of the basis capituli and firm support to the bases of the mouth appendages; running through these thick walls, ventral to the buccal cavity, is a pair of narrow canals leading to the bases of a single pair of hairs situated on the base of the hypostome. Into the postero-internal angles of the buccal cavity the large cylindrical *salivary ducts* open (s.d.); these are readily recognised by the delicate trachea-like transverse striation of their walls. Opening on the floor of the buccal cavity at its posterior end is the *pharynx*, a longitudinally placed chitinous sac of peculiar shape, which extends backwards into the body-cavity, where it becomes suddenly constricted into the narrow oesophagus. The relation of these parts will be more perfectly understood by reference to the figure showing a schematised longitudinal section through the capitulum (Text Fig. 6, p. 170)[1].

II. The Capitulum of the Male.

(Plate XIV, Figs. 3 and 4.)

The capitulum of the male differs from that of the female in details only, but these are sufficient to necessitate a special description. The dorsal ridge of the basis capituli bears at either lateral extremity, a short backwardly directed process (cornua). The porose areas are absent, a few scattered pores of the ordinary type being present. The palps are shorter and wider in proportion to their length and more pointed at the apex. The number of hairs borne on the infra-internal margins of the second article of the palp is usually thirteen, three being carried on the third article, as in the female. The hypostome is narrower in proportion to its length and not so markedly spatulate: the teeth which beset its ventral surface are also more elongated and sharply pointed. The chelicerae agree in their general structure with those of the female, but the digit calls for special attention. The internal article is similar to that of the female; the external article is relatively smaller in comparison with the internal, and only bears three cusps on its external margin. The dorsal process is quite different from

sion of these outlines is misleading, in the fact that they might be taken to represent external or superficial features.

[1] Nuttall, Cooper and Smedley (1905) described the details of the structure and relationship of the buccal cavity and pharynx; it will be considered again in a future paper which will follow in this *Journal*.

that of the female; it consists of a large triangular process, the shape and disposition of which are seen by reference to the figure (Pls. XIV, Fig. 4 and XV, Figs. 5, 6).

III. The Capitulum of the Nymph.

(Pl. XIV, Figs. 5 and 6.)

The capitulum of the nymph is intermediate in its general appearance between that of the adult and the larva. The salient ridges, seen in the adult on the basis capituli, are fairly well-developed: the anterior portion of the basis capituli is wider in front than behind, and its dorsal surface is produced laterally into large triangular flattened processes with rounded extremities: the postero-lateral angles of the ventral ridge are produced into short rounded backwardly directed processes, which for descriptive purposes we have styled "*cornua*." As in the adult, the basis capituli shows a posterior neck-like constriction immediately behind the dorsal and ventral ridges.

The *hypostome* is less spatulate and is provided on its ventral surface with four rows of denticles, about nine to each row.

The *chelicerae* of the nymph appear to be similar in all respects to those of the female.

The *palps* differ from those of the adult tick. Four articles can be distinguished, but the second and third articles are more or less fused with one another. The first article is very small; is completely concealed within the basis capituli and less readily distinguished than in the adult. The second article is large, and is raised on its ventral, lateral, and dorsal surfaces as a prominent rounded ridge, which forms the pronounced external angle of the palp. The infra-internal margin of this article bears five hairs, flattened and feather-like as in the adult, but wider at their bases and more tapering at their extremities. The third article is conical and forms the extremity of the palp; it is almost completely fused with the second article, the only line of demarcation being found towards the internal margin of the dorsal surface, where a deep indentation in the contour of the edge indicates the commencement of a fissure which is produced round the palp on its dorsal side for a short distance only. A shallow depression runs round the lateral surface of the article, and this, together with the arrangement of the hairs on its proximal side, shows the line of fusion with the second article. The infra-internal margin of the third article carries a single feather-like hair. The fourth article is

similar in form to that of the adult and likewise implanted into an oval fossa on the ventral surface of the third article.

The *porose areas* are absent.

IV. The Capitulum of the Larva.

(Pl. XIV, Figs. 7 and 8.)

The capitulum of the larva differs from that of the other stages in the relatively small size of its palps and in the fact that it is shorter in proportion to its width. The basis capituli is divided on its dorsal surface into anterior and posterior portions by a salient ridge, but unlike the later stages no ridge is developed on the ventral surface, and in addition the posterior portion is not so constricted. As in the nymph, a considerable amount of fusion exists between the articles, so much so in fact that the only free article is the fourth, the first, second and third being solidly fused together.

The *hypostome* is smaller than that of the nymph and bears four files of about six denticles.

The *chelicerae* resemble those of the female and nymph in form, all the structures being fully developed as in the adult (Pl. XV, Fig. 3).

The *palps* are very short and conical, strongly curved laterally and deeply concave on their median faces; the lateral angles are not so pronounced as in the nymph. They show a considerable amount of difference from those of the stages already described; the tendency to fusion of the articles as already shown in the case of the nymph reaches a maximum. Article 1 is very small and is exceedingly difficult to define; it is completely hidden within the basis capituli: articles 2 and 3 are the largest, but it is impossible to say with certainty, whether the line of folding of the cuticle (shown by a line in Pl. XIV, Fig. 7) is the line of fusion of the article: article 4 is relatively large and is more terminal in position than in the later stages.

A single feather-like hair is carried on the infra-internal margin of the palp, and in addition to this all the others, with the exception of the stiff bristle-like hairs on article 4, are serrate on one or both sides, as shown in the figures.

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EXPLANATION OF PLATES.

PLATE XII. *Haemaphysalis punctata.*

Larva. Dorsal and ventral aspects. ×62.
Drawn from living specimen by J. Ford.

PLATE XIII. *Haemaphysalis punctata.*

Nymph. Dorsal. ×30.
Female, unfed. Dorsal. ×20.
Female, gorged. Dorsal. ×5. Detail of surface more highly magnified, to left.
Female, gorged. Ventral. ×5. Spiracle to right.
Male, unfed. Dorsal. ×15.
Male, gorged. Ventral. ×20.
Male, unfed. Posterior portion of ventral surface, showing appearance of festoons for comparison with those of the gorged male. ×20.
Drawn from living specimens by E. Wilson.

PLATE XIV. *Haemaphysalis punctata.*

Fig. 1. Capitulum of Female. Dorsal aspect. ×85.
Fig. 2. ,, of Female. Ventral aspect. ×25.
Fig. 3. ,, of Male. Dorsal aspect. ×110.
Fig. 4. ,, of Male. Median portion, with left palp. Ventral aspect. ×125.
Fig. 5. ,, of Nymph. Dorsal aspect. ×220.
Fig. 6. ,, of Nymph. Ventral aspect. ×220.
Fig. 7. ,, of Larva. Dorsal aspect. ×400.
Fig. 8. ,, of Larva. Ventral aspect. ×400.

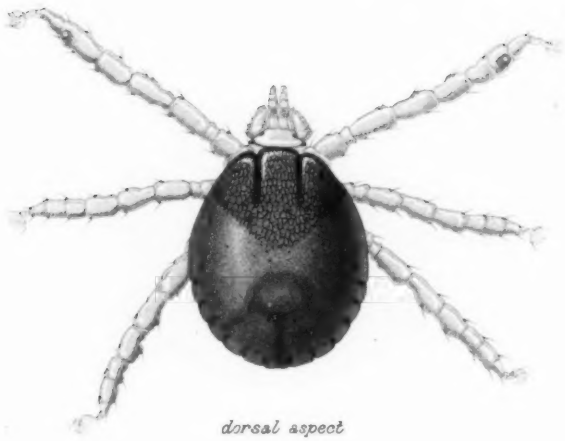
PLATE XV. *Haemaphysalis punctata.*

Fig. 1. Female. Tactile hairs on infra-internal margin of palp. ×250.
Fig. 2. Male. Ventral surface, showing details of external structures. ×30.
Fig. 3. Larva. Chelicerae, from ventral surface. ×1000.
Fig. 4. Female. Right chelicera, from ventral surface; sheath removed. ×250.
Fig. 5. Male. Left chelicera, from ventral surface. ×250.
Fig. 6. Male. Right chelicera, lateral aspect; sheath removed. ×250.

PLATE XVI. *Haemaphysalis punctata.*

Female. Transverse sections through the capitulum showing the relationships of the chitinous structures. ×125.

Fig. 1. Transverse section at level of digits of chelicerae (chelicera retracted).
Fig. 2. ,, ,, ,, ,, ,, distal third of hypostome.
Fig. 3. ,, ,, ,, ,, ,, proximal third of hypostome.
Fig. 4. ,, ,, ,, ,, ,, base of hypostome.
Fig. 5. ,, ,, ,, ,, ,, insertion of palps.
Fig. 6. ,, ,, ,, ,, ,, base of palps.
Fig. 7. ,, ,, ,, ,, ,, anterior margin of porose areas.
Fig. 8. ,, ,, ,, ,, ,, centre of porose areas.
Fig. 9. ,, ,, ,, ,, ,, posterior margin of porose areas.

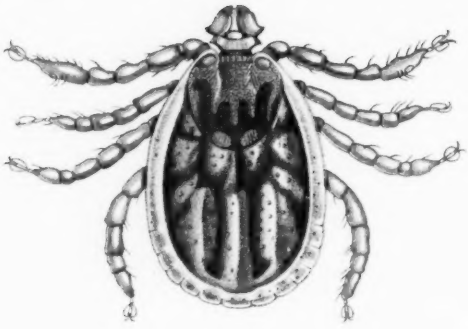


dorsal aspect

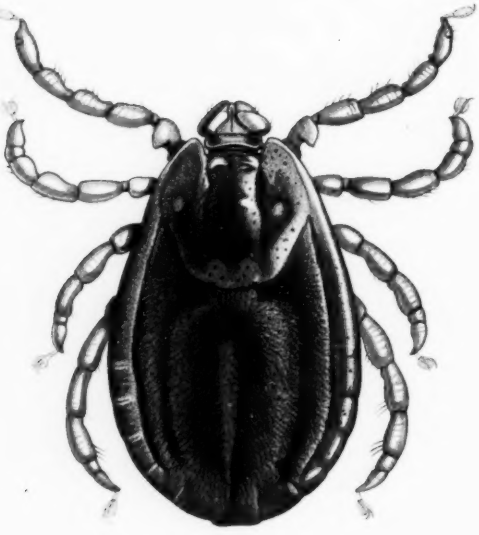
ventral aspect

E.Wilson, Cambridge

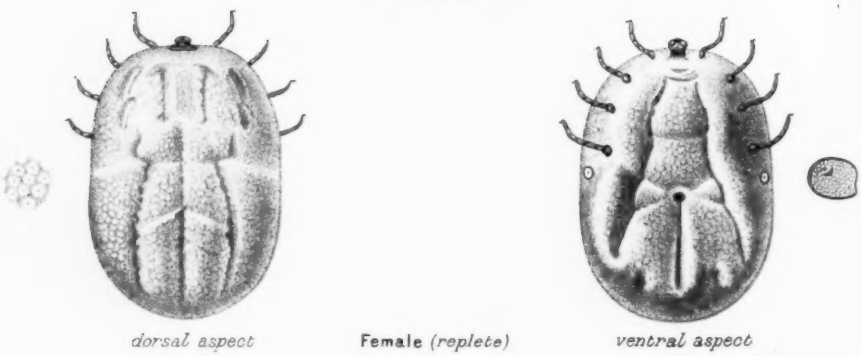
HAEMAPHYSALIS PUNCTATA Can & Fan.
THE LARVA.



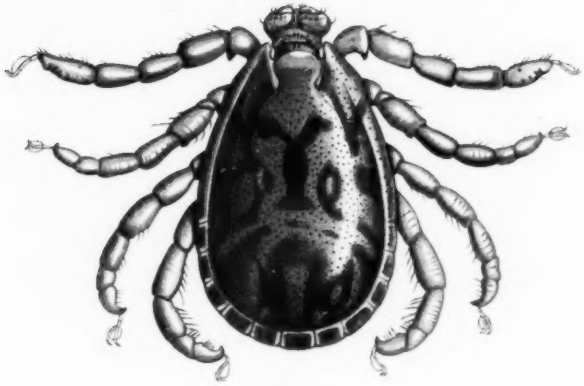
Nymph



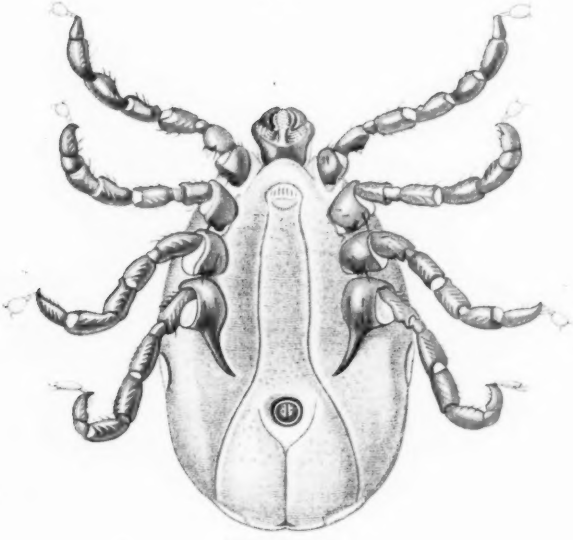
Female *(unfed)*



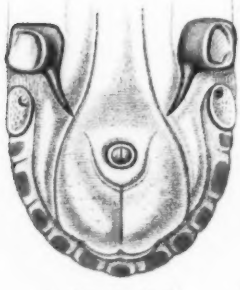
dorsal aspect Female *(replete)* *ventral aspect*



Male



Male *(replete)*



Male *(unfed)*

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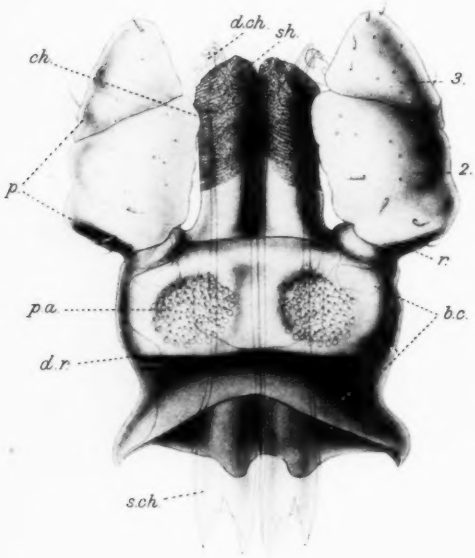
IS PUNCTATA Can. & Fan.



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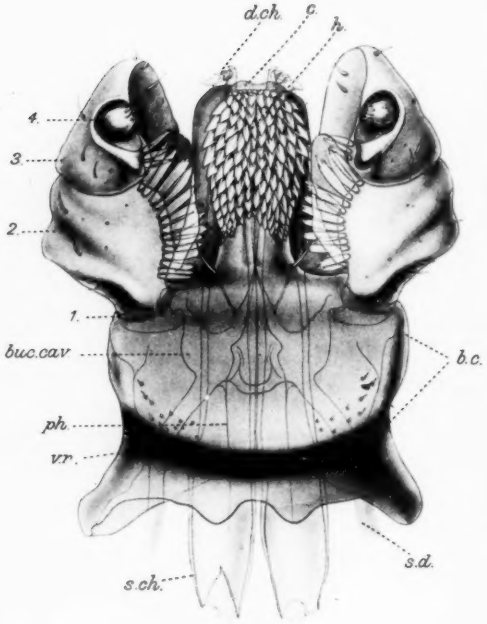

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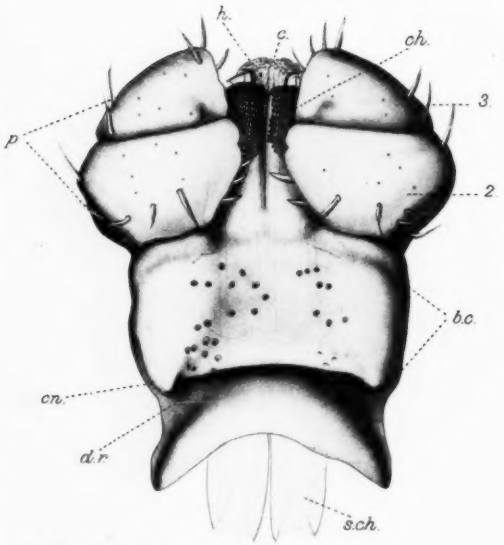

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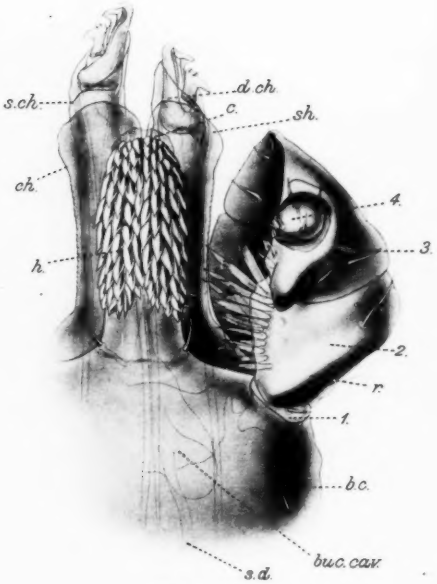
Fig.3.



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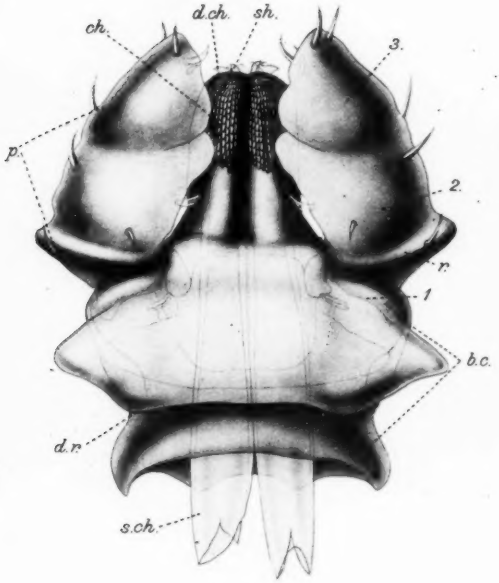

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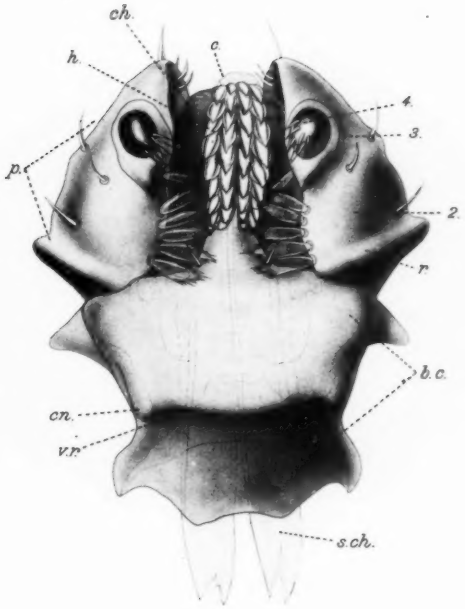

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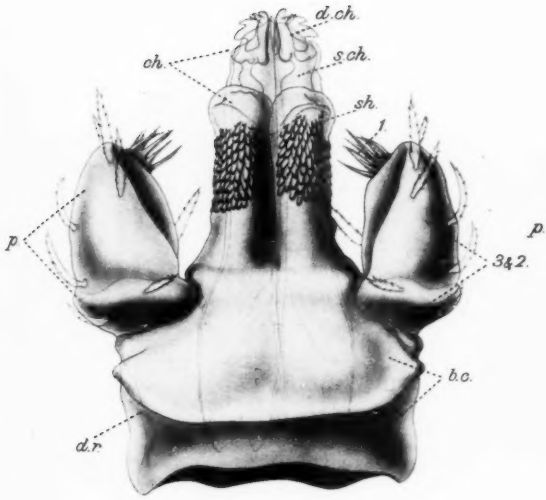

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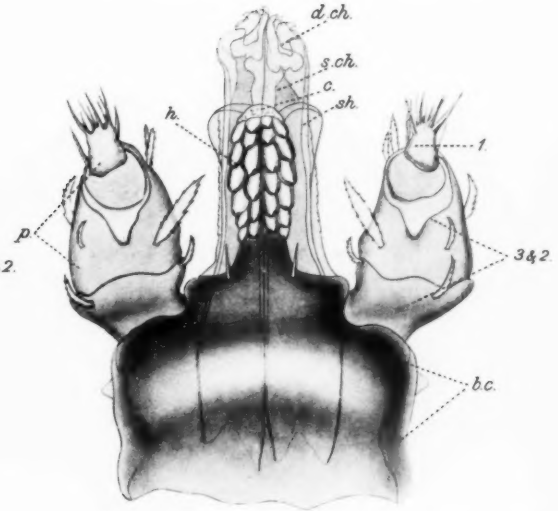
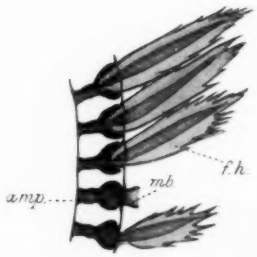


Fig.8.

Morgan & Kidd Richmond Collotype.



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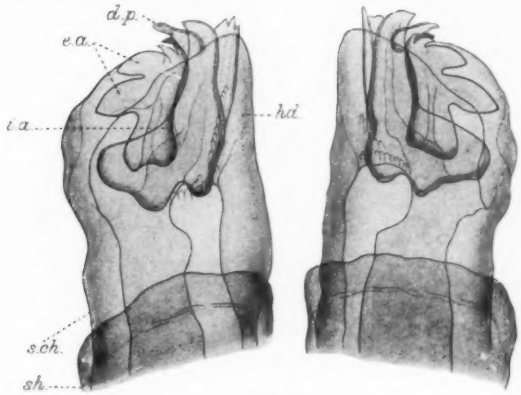

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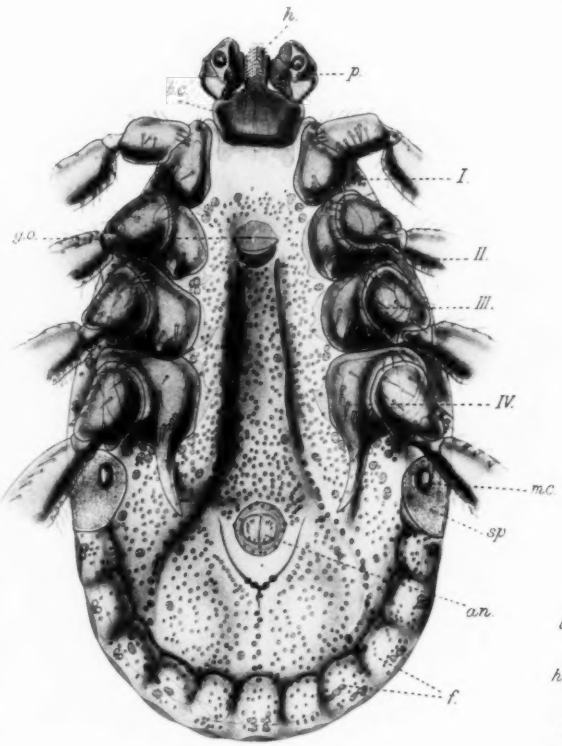

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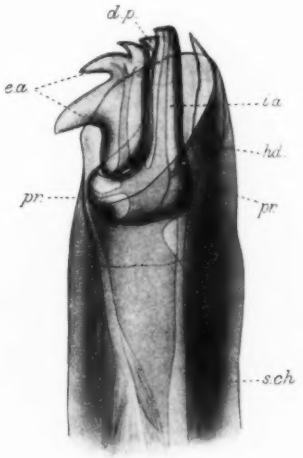

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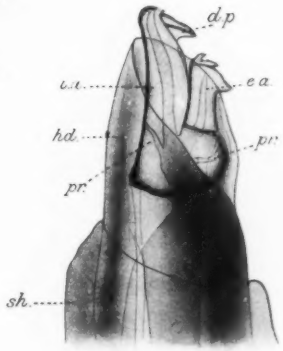

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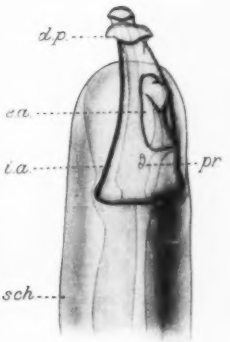

Fig. 6.

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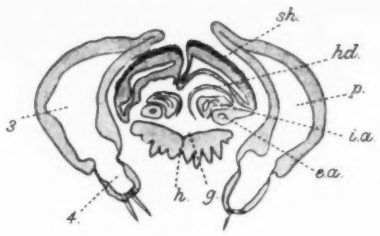
Morgan & Kidd Richmond Collotype.



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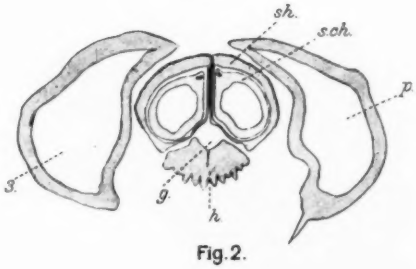

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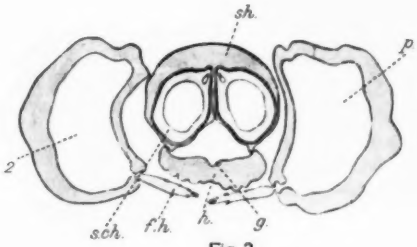

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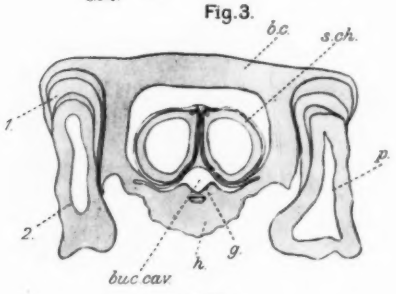

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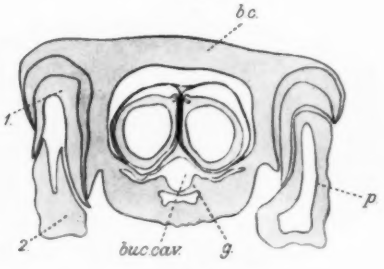

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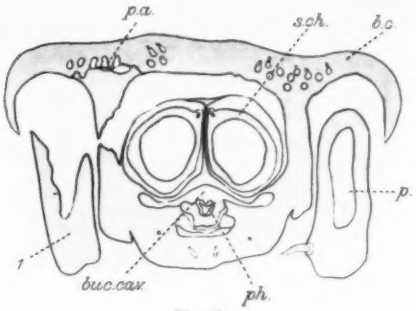

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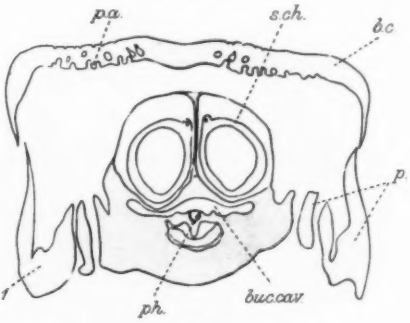

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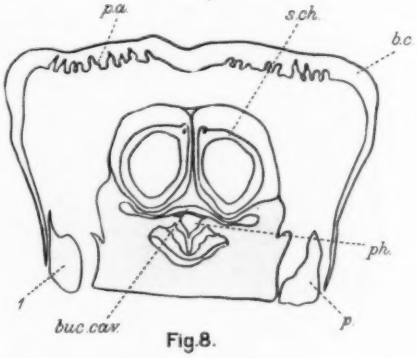

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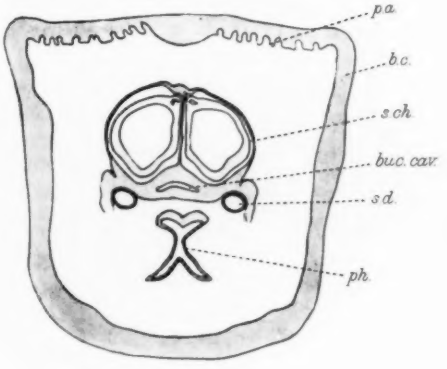

Fig.9.

L.E.R. del.

Morgan & Kidd Richmond Collotype.

INDEX TO LETTERING ON PLATES XIV—XVI.

amp.	ampulla at base of hair.
an.	anus.
b. c.	basis capituli.
buc. cav.	buccal cavity.
c.	corona.
ch.	chelicera.
cn.	cornu.
d. ch.	digit of chelicera.
d. p.	dorsal process.
d. r.	dorsal ridge.
e. a.	external article of digit.
f.	festoons.
f. h.	feather-hair on infra-internal margin of palp.
g.	gutter on dorsal surface of hypostome.
g. o.	genital orifice.
h.	hypostome.
hd.	hood surrounding digit.
i. a.	internal article of digit.
mb.	flexible membranous portion of feather-hair.
p.	palp.
p. a.	porose area.
ph.	pharynx.
pr.	pores on article of digit.
r.	ridge on article 2 of palp.
s. d.	salivary duct.
sh.	sheath of chelicera.
s. ch.	shaft of chelicera.
v. r.	ventral ridge.
1. 2. 3. 4.	articles of palps.
I. II. III. IV.	coxae.

HIRUDINEA AS HUMAN PARASITES IN PALESTINE.

By E. W. G. MASTERMAN, F.R.C.S., D.P.H. Camb.

LEECHES are common in the fountains and pools of Palestine, particularly in the northern parts, known to us familiarly as 'Galilee,' and further north in the district of the Lebanon. In the later summer and autumn months they are so plentiful in places that almost every horse and mule passing through these parts has a bleeding mouth. Under such conditions it is not wonderful that human beings from time to time are attacked. The domestic supplies of water are usually protected by the water-carrier's custom of straining it through a piece of fine muslin as he or she fills the pitcher. In some parts of the land the water at the source is kept free from leeches by means of fish; at Deishun, for example, a village in 'Upper Galilee' inhabited by Algerian settlers, the large stone tank into which the spring runs is full of a special fish—*Capoëta fratercula*, a kind of carp—which is there bred for the purpose and which is treasured by the inhabitants with superstitious reverence. Many of the springs in the mountains of Galilee and in the Lebanon are widely known for the multitude of leeches which lurk in their waters, but a thirsty traveller seldom has self-control enough to restrain himself from drinking, and when he does so, particularly at dusk or in the night, he is very likely to suck one in. It is stated in Allbutt and Rolleston's *System of Medicine* (2nd ed. Vol. II. Part II. p. 959) that the leech "remains in the stomach for a time and then begins to wander." I have had a long experience of leeches as human parasites but have never seen nor heard of a case like this. The leech in every case I have met has attached itself to the mouth, throat, etc. in the process of swallowing, and I am convinced that once the leech reaches the stomach it is killed. I have never heard of any after symptoms from cases where to my knowledge a leech has been completely swallowed. Many patients can describe the actual moment

(during deglutition) when the leech seizes on the throat. One would suppose from the great frequency of leeches in the mouths of horses, etc., that they must sometimes find a temporary resting place there in the human subject; such cases do not however come into the hands of a medical man—the patient or his friends readily removes such an intruder. In the cases, some three dozen or so, which I have had under my own observation the leech has been attached to the epiglottis, the pharynx, the nasal cavities, or—most common of all—the larynx. The frequency of the last situation in my clinical experience is doubtless due to the inability of the *fellahin* to do anything themselves for the relief of such cases.

The characteristic symptom produced by *Hirudinea* is the occurrence of constant small haemorrhages from the mouth or nose. Last summer in Safed I had a baby of about seven months brought to me with a history of almost continuous slight epistaxis from the left nostril of a week's duration. The child had been brought a considerable distance for treatment. On examining the nostril I noticed something blackish a little way up, and introducing a pair of fine sinus forceps I had the satisfaction of drawing out a slender leech about $1\frac{1}{2}$ ins. long. The symptoms were at once relieved. The first case I had, on September 16, 1893, also at Safed, was remarkable. The patient, a girl of 12, came complaining of constant small haemorrhages from the mouth for three days; there was no dyspnoea nor aphonia. I put my right index finger well to the back of the tongue to see if I could feel anything abnormal in the neighbourhood of the epiglottis, and when I drew it forth was astonished to find a small leech looped up upon my finger-nail. These are mild cases. When the leech lies deeper the symptoms are much more distressing. The haemorrhage, though never great in quantity at any one time, may, when prolonged, be very serious and even fatal. I know of two cases where the patient actually died from this. One, which occurred shortly before I came to Palestine, was narrated to me by Dr Torrance of Tiberias. The man came under medical treatment already reduced to extreme anaemia by prolonged haemorrhage; all efforts failed to reach the leech and it was only after complete eversion of the patient that it was at last extruded, but the patient never recovered the loss of blood. The second case is that of a young fellah girl who was brought to Safed last summer for the removal of a leech which she had harboured in her throat for some weeks. She just reached Safed before she died as a result of anaemia.

The haemorrhagic discharge is pure blood, bright in colour and,

when the leech is in the pharynx or larynx, it is 'hawked up' almost continuously. As a rule, and of course in all laryngeal cases, there is considerable irritation about the larynx resulting in a short irritating cough, dyspnoea, varying in intensity from time to time, and sometimes considerable cyanosis from impeded respiration. The patient's expression is one of considerable distress, a great contrast to that following upon the instant relief afforded by the removal of the parasite. In laryngeal cases there is often complete loss of voice, the patient only speaking in a whisper and then with difficulty. On examination with a tongue depressor the leech is sometimes revealed, lying at the back of the pharynx; in such a situation it cannot be mistaken as its black, rounded, annulated, shiny outline at once catches the eye. More commonly it is necessary to use the laryngoscope, and in a considerable proportion of cases the leech will be found attached close to one of the vocal cords. Its head attachment is sometimes just inside or just outside the cord, and it flops in and out of the aperture with the respiration. In such cases the wonder is not that the patient suffers from dyspnoea, but that he can breath at all. The upper larynx and the pharynx are largely bespattered with fresh blood and in the process of examination the patient usually coughs out a good deal of it. Why it is that a leech, which outside the body takes its fill and then drops off or is easily removed, continues when inside the mouth and throat to keep its hold, though apparently attacking different spots—leaving the old points to bleed—is a question I must leave to zoologists, but it is certainly the case, and in my experience in such a situation it seldom voluntarily quits its hold. According to the *System of Medicine* quoted above this species of Hirudinea is *Limnatis nilotica.*

As regards the treatment, the natives of Palestine are accustomed if the worm is within their reach to transfix it with a sharp thorn from the *Sidr* tree: I have seen muleteers use a packing needle to extract a leech from a mule's mouth. When the parasite is beyond reach of this process they use what they call *es salata,* that is the thick deposit which collects in their tobacco pipes. This they smear on the end of a fragment of wood and they say that if this touches the leech it is poisoned and leaves its hold. The cases which came to the European practitioner are almost always those where the skill of the amateur has failed and for these such simple means are useless. In my experience too the 'spraying with salt solution' mentioned in Allbutt and Rolleston's *System* has proved of little use. The two means I have found most successful are either mechanically seizing the worm by means of suitable forceps or paralysing it with cocaine.

In a certain proportion of cases it is possible to seize the leech. With pharyngeal cases this should be the rule and perhaps with throat specialists it might also be the case with laryngeal cases. For the ordinary surgeon, though it is simple enough to watch the worm through laryngoscope mirror, it is very difficult to seize the writhing, slippery creature amidst the spasmodic contractions of the larynx and the frequent coughing. Spraying with cocaine is a great help, but I must admit that in mechanically removing the leech I have several times had failures. The second method, which I have never yet known to fail, I owe to the suggestion of Dr Mackinnon of Damascus—this is the application of a strong solution of cocaine to the parasite. It is not enough to spray in cocaine or paint it indiscriminately. For sure success a small pledget of cotton-wool, saturated with a 30 % cocaine solution, should be actually brought in contact with the parasite by carefully directed movements guided by the help of the laryngoscopic mirror. The strong cocaine solution, if it reaches the surface of the leech undiluted always produces in a few minutes—*not*, in my experience, instantaneously—a paralysing effect, causing it to relinquish its hold. My last three cases, all treated in this way, were speedily successful. In the case of one of these patients the man apparently swallowed the leech for he had no more symptoms from a few minutes after treatment; in the second case the leech was suddenly coughed into the mouth about five minutes after the cocaine application and I removed it from the mouth with my finger. The last case was one I found in our Hospital in Jerusalem immediately on my return from Safed. The man had for nearly a week been 'spitting blood,' and when I was called to see him a spittoon full of practically pure blood was lying on the locker by his side and every few minutes he was adding more: his lips were cyanosed; he was unable to speak above a whisper and every few seconds he had a short cough. I sprayed cocaine solution upon the fauces and the laryngeal mirror at once revealed a large leech attached close to one of the vocal cords. After several vain efforts to seize it with curved forceps I applied cocaine as described and then made the man lie prone upon his bed with his head hanging over the edge. After a few minutes he coughed up the leech on to the floor. I have heard it stated that there is a danger that the parasite on leaving its hold may fall downwards and lodge on one of the bronchi, then die and set up septic changes. I have never seen such a case, but such a danger is doubtless obviated by making the patient assume the position I have described when the cocaine is commencing to have its effect.

NOTE ON A GNATHOBDELLID LEECH [*LIMNATIS* SP.?] FROM ANGOLA.

By W. A. HARDING, B.A.

I owe to Prof. G. H. F. Nuttall and Mr A. E. Shipley the opportunity of examining three specimens of a West African leech sent by Dr C. Wellman from Benguella, Angola. They were described as land leeches 'taken four or five miles from any water' and were preserved in alcohol. A brown, vermiform object protruded from the mouth of the largest specimen.

Transverse sections of this object revealed muscular tissue but gave no further indications of its nature. Many leeches [e.g., *Trocheta*; *Aulastoma*, Moq. Tand.] are known to leave the water voluntarily in search of food such as worms and molluscs, and it may be assumed with some certainty that this specimen possessed similar habits and had been killed in the act of devouring its prey.

Diagnostic characters.

The body resembles *Hirudo* in form, except that, anteriorly, it tapers rather more rapidly. The following measurements were taken:

Largest specimen: 51·4 mm. long and 9·7 mm. in width.

Smallest specimen: 26 mm. long and 5·60 mm. in width.

The acetabulum is circular and of moderate size.

The colour is brownish-grey without any trace of pattern or coloured spots, rather paler on the ventral surface. There is an ill-defined, median, dull yellow tract on the dorsal side, following the course of the alimentary canal.

Eyes 10; arranged, as in *Hirudo*, on the 1st, 2nd, 3rd, 5th and 8th annuli [Fig. 2]. The first three pairs are distinct, the fourth pair less apparent, the fifth pair almost imperceptible. They are best seen on the smallest specimen.

Annuli 102; counting from the first pair of eyes to the acetabulum. The 5th and 6th [the buccal annuli of Whitman] are united ventrally to form the first ventral ring; the 6th and 7th are united ventrally to form the second ventral ring [Fig. 1]. The annuli have a shallow, median wrinkle, parallel to their sides, which

is sometimes split up into subsidiary wrinkles. This feature may be due to contraction in alcohol.

The large and prominent anus lies in the 102nd annulus, causing it to become double [Fig. 4].

The genital apertures are five rings apart, small and difficult to see. The male orifice lies between the 31st and 32nd annuli; the rather larger female orifice lies between the 36th and 37th annuli.

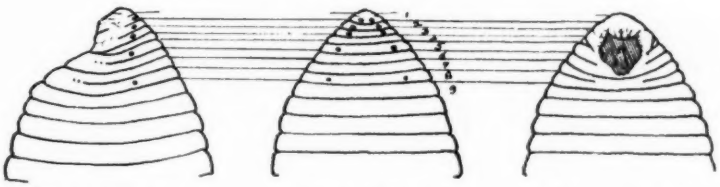

Fig. 1. Side view. Fig. 2. Dorsal. Fig. 3. Ventral.

Diagrams showing eyes and anterior rings.

The horizontal lines connecting the figures define the annulations which are numbered 1—9 in the middle figure (annulation 1, indicated by a broken line, was indistinct).

Fig. 4.

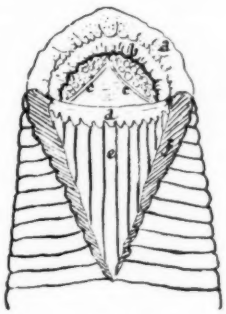

Fig. 5.

Fig. 4. Dorsal aspect of posterior rings and acetabulum.

Fig. 5. Diagram of ventral surface of anterior sucker.

a. Outer lip.
b. Its inner, crenulate border.
c.c. The diverging ridges.
d. Edge of mouth with frilled membrane.
e. Interior of pharynx.
f. Cut edge of body, opened by a median ventral incision.

The general characteristics of the leech indicate that the complete somite is composed of five annuli. As no segmental papillae or nephridial pores can be detected [owing to immersion in alcohol?] it is impossible to determine this point with certainty from external examination.

The crop, which was not fully investigated posteriorly, has paired caeca like *Hirudo*.

The blood is red.

The pharynx is long and plicated as in *Aulastoma*, Moquin Tandon. In the largest specimen these plications or folds are indistinct; in the second specimen twelve, equal, longitudinal plications were clearly seen.

The ventral side of the anterior sucker is bounded anteriorly by a deeply wrinkled lip with an inner crenulate border. From a median point slightly behind this border, two ridges diverge posteriorly, dividing the hollow, ventral surface of the sucker into three depressions and forming a triangle with the edge of the mouth, which appears as a transverse ridge in individuals laid open by a median, ventral incision as shown in Fig. 5. The depressions within and without this triangle vary considerably in form according to the amount of stretching to which the individual is subjected, a fact which may account, to some extent, for the conflicting descriptions of similar features in other leeches.

The ridge formed by the edge of the mouth is extended posteriorly into a frilled membrane overhanging the ends of the pharyngeal plications and beneath it the maxillae, if present, should be found.

Although the above diagnostic characters are not complete, it is evident that Dr Wellman's leech is one of the *Gnathobdellidae*, a group which Dr R. Blanchard divides into the two sub-families *Hirudininae* and *Haemadipsinae*.

The presence of a ring between the third and fourth pairs of eyes is sufficient to separate this leech from the *Haemadipsinae* or true land leeches, and it therefore must be placed in the first sub-family.

The same authority assigns to the *Hirudininae* the following genera, viz., *Hirudo*, *Haemopis* [= *Aulastoma*, Moquin Tandon], *Limnatis*, *Hirudinaria*, *Macrobdella*, *Whitmania* and *Limnobdella*.

This leech differs, amongst other characters, from *Hirudo* in the absence of the three powerful jaws; from *Haemopis* [= *Aulastoma*, M.T.] in the form of the crop; from *Hirudinaria* and *Macrobdella* in the number of rings separating the genital apertures. I am unable to speak positively with regard to *Limnobdella* and *Whitmania* as I have been unable to analyse somites VI and XXIII owing to the absence of segmental papillae. Taking everything into consideration I venture to place Dr Wellman's leech in the genus *Limnatis* which it appears to resemble most closely. In particular, Dr Blanchard's description of the anterior sucker of *Limnatis* as "creusée d'un sillon en dessous" and Moquin-Tandon's description of the same as "profondément creusée en dessous d'un canal en triangle" seem to agree with the characters shown in Fig. 5.

[N.B. It is to be noted that Professor Blanchard agrees with Leuckhart, Whitman and Apáthy in rejecting *Haemopis*, Moquin Tandon, 1846, as unworthy of generic rank.

He substitutes *Haemopis*, Savigny, 1817 for *Aulastoma*, Moquin Tandon, 1826; and *Aulastoma gulo*, Moquin Tandon, 1846, becomes *Haemopis sanguisuga*, Bergmann, 1757.]

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NOTE ON *CYSTIDICOLA FARIONIS* FISCHER. A THREAD-WORM PARASITIC IN THE SWIM-BLADDER OF A TROUT.

By A. E. SHIPLEY, M.A., Hon. D.Sc. (Princeton), F.R.S.,
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CYSTIDICOLA FARIONIS. G. Fischer, 1798.
Synonyms: *Fissula farionis.* Bosc, 1802.
Fissula cystidicola. Lamarck, 1801.
Ophiostoma cystidicola. Rudolphi, 1809.
Spiroptera cystidicola. Rudolphi, 1819.
Dispharagus cystidicola. Dujardin, 1845.
Ancyracanthus cystidicola. Schneider, 1860.
Ancryacanthus cystidicola. von Linstow, 1878.

Some hundred and ten years ago, Dr Gotthelf Fischer, in a communication dated from Vienna, described a Nematode living in the swim-bladder of the trout. He named this worm *Cystidicola farionis* and his description is accompanied by some rough cuts. Bosc (1802) re-described the same Nematode under the name *Fissula farionis.* The same worm was again described by Rudolphi (1819) under the name *Spiroptera cystidicola.* Dujardin (1845) mentions it under the name *Dispharagus cystidicola.* Later it was removed by Schneider (1860) from the genus *Spiroptera* and placed in Diesing's genus *Ancyracanthus.* von Linstow uses this generic name "with a difference"; he spells it *Ancryacanthus.*

Ramsay Wright (1879) records the species from *Salmo siscowet* Agassiz. It is widely spread on the continent of Europe and is now—I think for the first time—recorded in England.

Mr R. T. Leiper of the London School of Tropical Medicine, who kindly examined some specimens of these round worms which I had recognized as belonging to Schneider's species *A. cystidicola,* has pointed out that the worms were originally described and figured by G. Fischer and that his name *Cystidicola farionis* still stands. Mr Leiper has written a note on the anatomy of the worm and his description follows (see p. 193).

Certain trout found in the streams on the estate of the Hon. Sydney Holland, Royston, Herts., have been dying off in considerable numbers during the spawning season for the last year or two. Some of these were sent to Cambridge this spring, and, on dissection, it was found that the cavity of the swim-bladder was infested with Nematode worms of the species described under the name *A. cystidicola* by Schneider, now re-called *C. farionis*.

In his original description Fischer draws attention to the curious atmosphere it which the worm lives. He describes the gas in the swim-bladder of the trout as being almost pure nitrogen with a little CO_2. Whether a more recent analysis of the gas from this fish's swim-bladder has been made I do not know but as a rule in fresh-water fishes, to quote Günther, the gas "consists of nitrogen with a very small quantity of oxygen and a trace of carbonic acid." The proportion of oxygen is usually greater in sea-fishes and it also varies in the same fish at different seasons. Bunge (1890) has shown that *Ascaris mystax* from the intestine of the cat can live for four or five days in an atmosphere quite free from oxygen and that in a similar medium *A. acus* from the pike will live and move for from four to six days. Probably the traces of oxygen in the trout's swim-bladder are sufficient to supply the meagre wants of *C. farionis*.

The parasites are stated to be more numerous in the winter: in the late spring they seem to average 8 to 18 or so in each fish. They have also been met with in the oesophagus and probably enter the swim-bladder through the "ductus pneumaticus."

The question whether the presence of these parasites is injurious to the fish is a debateable one. They are usually regarded as harmless. Certainly many of Mr Holland's trout have been dying but the worms were found in the trout before the mortality set in, and in those fish we examined at Cambridge they were at least as numerous in fish reported as being healthy as in those fish that had died. On the other hand all the dying fish appear to suffer from some derangement of the swim-bladder; they swim always on the surface on the water and die with their heads downwards and the body almost if not quite perpendicular to the surface of the water. They die, as Mr Holland tells me, not only in deep water but occasionally in the shallow water of the spawning beds. This year however during the period of greatest mortality when 6 or 7 trout were dying a day until some 50 out of 200 were dead, they never tried to work up stream to the spawning beds but died in the deep water. The fish seemed to have difficulty about shedding their ova and it may be

that this difficulty is connected with the cause of the mortality, but males died with the same symptoms as females, though not in such large numbers. Since the spawning season finished this year there have been no deaths. Curiously, too, the rainbow trout are alone affected and not the brown trout.

I am indebted to Mr Holland not only for the Nematodes but for many of the facts mentioned in this note.

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NOTE ON THE ANATOMY OF *CYSTIDICOLA FARIONIS.*

By R. T. LEIPER, M.B., F.Z.S.
Helminthologist to the London School of Tropical Medicine.

The nematode worms discovered by Mr Shipley in the swim-bladder of Trout belong to the species *Cystidicola farionis*, first described by Fischer, and considered by him to represent a new and distinct type in nematode structure for which he proposed the generic name *Cystidicola*. Later writers refused to accept the new genus and included it in various other groups (see Synonymy, p. 190). In accordance with an old nomenclatural practice the proposed generic name was adopted as the specific name in place of that originally given, thus *Cystidicola farionis* became, for Rudolphi, *Spiroptera cystidicola*. From a detailed examination of the various characters of *C. farionis* I have come to the conclusion that Fischer was correct in creating a new genus for the new forms, and I therefore propose the reinstatement of the abandoned name *Cystidicola*. It is also in accordance with modern nomenclatural ruling that the original specific name *farionis* should be adopted in place of *cystidicola*.

The anatomy of C. farionis.

The body is very slender and translucent, it tapers gradually from the middle third to both ends of the body. In preserved specimens the male is coiled posteriorly in a spiral manner and measures about 14 mm. in length. The female remains perfectly straight, measuring 22—26 mm. × ·3 mm. The cuticle is thin and without transverse striation. At the posterior end of the male it expands to form a narrow ala on either side supported by the genital papillae. In the female its uniformity is disturbed only by a tiny pair of papillae near the tip of the tail.

The alimentary canal shows the usual differentiation into oesophagus, chyle intestine and rectum, the first-named structure presenting characters that associate *Cystidicola* with the other genera of the family Spiruridae. The mouth is a simple spherical orifice surmounted by two hook-like teeth that in shape recall the "external tooth" of *Physaloptera* species. It gives access to a deep cylindrical mouth capsule with thick

chitinous wall measuring ·1 mm. × ·01 mm. The oesophagus is exceedingly long and narrow; it measures about 2·7 mm. × ·5 mm.; the anterior fifth is narrower than the succeeding portion and does not contain muscular fibres. The nerve ring encircles this slender part at its middle, *i.e.* about ·3 mm. from the anterior end of the body. The male genital system presents the following features: the testicular tube extends forwards without a single convolution from the cloaca along the ventral surface of the chyle intestine to end blindly at the junction of the anterior and posterior halves of the body. There are two spicules, unequal in size and thickness; the long slender spicule measures ·75 mm., the short one ·16 × ·025 mm. The cloaca opens ·2 mm. from the tip of the tail and is enclosed on either side by a narrow cuticular ala extending from the end of the body along each lateral edge to a similar distance in front of the cloacal orifice. Within the alae are seen a double series of nipple-shaped papillae, there being nine pairs of prae-anal papillae and five single post-anal papillae in each ala. The components of the most anterior pair of prae-anals are considerably separated, whilst those of the other pairs lie close together, one slightly internal to the other.

The female genital system opens by a very short vagina in the centre of the ventral surface, midway between the head and tail.

From the vulva the vagina passes backwards, not forwards as stated by Schneider, for a distance equal to the diameter of the worm at that level and there divides into two uterine tubules, one of which continues directly backwards for a distance equal to a fourth of the body-length, doubling upon itself once in this distance in a coil of half that length and is then continued as an ovarian tubule. The other uterine tubule turns abruptly forwards from the vagina and describes a very similar course in the anterior region of the perivisceral cavity. Both ovarian tubules make two or three short coils. Those of the anterior tube only slightly overreach the junction of the oesophagus with the intestine, those of the posterior one do not extend so far as to encroach upon the posterior sixth of the body.

The mature female is ovoviparous, the eggs have thick shells with a curious tuft of exceedingly delicate filaments, two or three in number, attached to a small cuticular knob at either pole. The eggs measure ·05 × ·025 mm. and the shell is ·005 mm. thick. The only other nematode in which such filaments have been noted, to my knowledge, is *Ascarophis morrhuae*, van Beneden (1870)[1], in which species there are cuticular appendages at one pole only.

[1] van Beneden (1870), *Les poissons des côtes de Belgique*, Plate III. fig. 11.